



PHD

**Influence of Habitual Smoking on Physiological Status, Physical Performance
Adaptation and Injury Risk during Initial Military Training**

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Award date:
2013

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University of Bath

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Influence of Habitual Smoking on Physiological Status, Physical Performance Adaptation and Injury Risk during Initial Military Training

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A thesis submitted for the degree of Doctor of Philosophy

University of Bath

Department for Health

SEPTEMBER 2012

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Abstract

Cigarette smoking has been reported to be prevalent in military training populations, and associated with lower cardiorespiratory fitness and higher risk of training-related injury. However, it is unclear whether habitual smoking impairs development of physical fitness. It is possible that smoking-induced alterations in oxidative stress, inflammation and hormone balance may disrupt training adaptation in smokers. The aim of this programme of work was to identify the influences of smoking on physical performance adaptation, selected biomarkers and injury risk in a military trainee population. The first study established that habitual smokers comprised 48% of a cohort of 2087 trainees. Upon closer examination, both at entry (Study 2) and during 10 weeks of training (Study 3) smokers exhibited chronically elevated oxidative stress and, after commencement of training, evidence of significantly higher resting inflammation compared with non-smokers. Throughout the full duration of training, performance of smokers in military physical fitness tests was significantly worse than non-smokers (Study 4), but neither muscular adaptation nor physical performance improvement were impaired in smokers in the early stages (10-14 weeks) or over the full duration (26 weeks) of training. It was expected that smokers would experience greater acute inflammatory responses to exercise but neither these, nor hormonal responses, differed between smokers and non-smokers in response to consecutive days of military field exercise (Study 5). In addition to poorer physical performance in smokers, training-related injury incidence was higher in smokers than non-smokers, specifically injuries attributed to overuse (Study 6). Overall, smoking appears to cause some physiological alterations which, while not impairing adaptation to training, may have adverse implications on health outcomes. Although the specific underlying mechanisms are unclear, habitual smokers exhibit greater injury risk and typically lower physical fitness than non-smoking counterparts.

Acknowledgements

I must express my gratitude to the many people who have contributed to my being able to complete the work towards this thesis.

Firstly, I must extend great thanks to my supervisors, for sharing their experience and knowledge, and their generosity with their time. To Dr Keith Stokes, for his invaluable input to shaping my understanding of science; improving every aspect of my scientific writing and the nature of my critical thinking; the countless hours of guidance and discussion on tough decisions; and for teaching the importance of simplicity. To Dr James Bilzon for his support with all things military; the opportunities he has created for me and his generosity in supporting my development whilst building the framework for this research. Thirdly, to Dr Dylan Thompson for always being free to answer the contentious questions, and teaching me to have a holistic approach to science.

This research would not have been possible without the funding and support of the Army Recruiting and Training Division, Ministry of Defence. Special thanks must also go to Dr Pedro Tauler Riera, Univeritat de les Illes Balears, for completing the analysis of oxidative stress, and Mark Robinson for construction and validation of the lifestyle questionnaire. Further thanks must go to members of the research team who devoted hours of time to help with data collections, including Dr Oliver Peacock, Tom Nightingale, Oly Perkin and Rachel Izard. I also extend thanks to the trainees who gave up their time to take part in the research, without whom all this would not have been possible.

To fellow postgraduates, staff and friends in the department. Great thanks must go to Enhad for always having the time for discussion whether academic or non-academic when sorely needing clarity. Similar thanks must go to JP, alongside his early guidance on biochemical analysis and for fashioning me into a practical scientist. Also, thanks to Ros and Tim for the well needed breaks and opportunities for the consumption of endless rounds of coffee.

Lastly, thanks must go to my family, for their constant, undivided support through every step, and to my friends and those close to me over these past years, for always believing in me, instilling motivation, listening and supporting at every turn.

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Abbreviations

ALT	Alanine Transaminase
ANOVA	Analysis of Variance
ATR	Army Training Regiment
BHT	Butylated Hydroxytoluene
BMI	Body Mass Index
CI	Confidence Interval
CIC	Combat Infantryman's Course
CRP	C-reactive Protein
FER	Forced Expiratory Ratio
FEV₁	Forced Expiratory Volume (in one second)
FS	Former Smokers
FVC	Forced Vital Capacity
HB	Haemoglobin
HCT	Haematocrit
HPLC	High Performance Liquid Chromatography
HS	Heavy Smokers/Heavy Smoking Group (≥ 20 cigarettes per day)
<i>I</i>	Injury Incidence
IGF-1	Insulin-like Growth Factor-1
IL-6	Interleukin-6
IQR	Inter-quartile Range
ITC(C)	Infantry Training Centre, Catterick
LOOH	Lipid hydroperoxides
LS	Light Smokers/Light Smoking Group (1-9 cigarettes per day)
MCH	Mean Corpuscular Haemoglobin
MCV	Mean Corpuscular Volume

MDA	Malondialdehyde
MPQ	Military Pre-training Questionnaire
MS	Moderate Smokers/Moderate Smoking Group (Between 10-19 cigarettes per day)
MTSS	Medial-tibial Stress Syndrome
NS	Non-smoker/Non-smoking group
OR	Odds Ratio
OS	Occasional Smokers
PEF	Peak Expiratory Flow
PFT	Physical Fitness Test
RBC	Red Blood Cell
ROS	Reactive Oxygen Species
RR	Relative Risk
S	Habitual Smokers/Smoking group (>1 cigarette per day)
SD	Standard Deviation
SE	Standard Error
SHBG	Sex Hormone Binding Globulin
TAC	Total Antioxidant Capacity
TBA	Thiobarbituric Acid
TEP	Tetraethoxypropane
TNFα	Tumour Necrosis Factor α
$\dot{V}O_2$ max	Maximum oxygen uptake
WBC	White Blood Cell

CHAPTER 1

General Introduction

Introduction

It is widely established that cigarette smoking adversely impacts health and increases risk of numerous chronic diseases (Zeiher, Schächinger & Minners 1995; Ambrose & Barua 2004; Doll et al. 2005; Birrell et al. 2008). Owing to its associations with chronic disease states, smoking is considered the greatest preventable cause of premature death worldwide (Fagerström 2002; World Health Organisation 2004). Regular smoking is also negatively associated with cardiorespiratory fitness (Bernaards et al. 2003) but this has only been widely recognised in middle-aged and elderly populations. Having received little attention in scientific research, it is not known whether smoking affects physical fitness in young, active populations or an individual's ability to improve physical fitness. The sparseness of research in this area may stem from there being relatively few populations that habitually smoke while also participating in a long-term physical training programme. Evidence from the United States and Scandinavia that military training populations have a higher proportion of smokers than the general public (Heir & Eide 1997; Klesges et al. 2001) suggests military trainees provide an appropriate platform to examine the potential effects of habitual smoking on physical fitness development in a young, physically active population.

In numerous American military populations, cigarette smoking has been associated with lower physical fitness and a higher risk of training-related injury (Altarac et al. 2000; Knapik, Sharp, et al. 2001; Ward et al. 2003; Haddock et al. 2007). However, differences between training environments, practices and training duration mean the findings from military populations abroad cannot be easily transferred to their British counterparts. To date, injury incidence and associated risk factors have been examined during deployment training of British servicemen (Wilkinson et al. 2011) but comprehensive study of injury and fitness in key British Army trainee populations have not been completed. The development of physical fitness from during initial military training is imperative for trainees to pass successfully into the armed forces and excel under the physical demands of military service. Therefore, greater knowledge of any adverse influence of smoking on improvement in physical performance would be beneficial to military organisations.

Despite this, it appears that only one study has examined the difference in performance adaptation between smokers and non-smokers, and was completed in British Army Officer cadets (Hoad & Clay 1992). In this study it was demonstrated that performance improvement to a 6 month training programme was significantly greater in non-smokers. To date, however, no other studies have been attempted to re-examine this hypothesis or improve on this research, whether in the military or in the general public. Additionally, despite extensive work in military populations, the underlying mechanisms induced by smoking that could mediate impaired adaptation to training or heightened injury risk still remain unclear.

Several avenues by which smoking could influence injury risk and recovery from exercise have been reported in previous research. Chronic differences in oxidative stress (Basu et al. 2009), inflammation (Helmerson et al. 2005) and circulatory hormone levels (Kapoor & Jones 2005) have been observed between smokers and non-smokers in middle aged and elderly populations. Reactive oxygen species (ROS) are contained and produced in cigarette smoke (Pryor 1997). Accumulation of ROS causes oxidative stress in lung tissue (Barreiro et al. 2010) that appears to have a concurrent effect on elevating markers of oxidative stress in the circulation (Morrow et al. 1995; Ahmadzadehfar et al. 2006). A persistent elevation in oxidative stress can be harmful to cell membranes, DNA (Nair et al. 1996) and functional components of cells (Coirault et al. 2007) by protein and lipid peroxidation. Increases in inflammatory mediators and immune cells are observed in lung tissue and in the circulation in response to a rise in oxidative stress and oxidative damage (Cross, Van der Vliet & Eiserich 1998; van der Vaart et al. 2004; Yanbaeva et al. 2007). Habitual smoking, both through the indirect actions of nicotine on endocrine glands, and via signalling from inflammatory cells, induces secretion and suppression of several circulatory growth factors and stress hormones (Kapoor & Jones 2005; Steptoe & Ussher 2006). It is possible that these effects are associated with smoking in a dose response manner, whereby increased cigarette consumption (cigarettes per day) induces greater changes.

The above processes have been found to be influential in the effective maintenance and recovery of muscle and bone from exercise (Basu et al. 2001), and healing of connective tissue (Jorgensen et al. 1998). Coirault et al. (2007) demonstrated that inducing an elevation of ROS in muscle cells caused oxidation of

myosin heavy chains and a reduction in muscle contractility. Similarly, the incubation of muscle tissue in inflammatory cytokine interleukin (IL)-6 induced protein breakdown (Goodman 1994). Both oxidative stress and low-grade systemic inflammation are implicated in disease-induced muscle atrophy (Schaap et al. 2006; Moylan & Reid 2007). Equally, growth factors promote the maintenance of muscle mass by protein synthesis or suppressing protein breakdown (Musrò et al. 1999; Musrò et al. 2001; Axell et al. 2006). Given the relationships described above, there is evidence that a disruption to these processes whether at rest or during exercise training could be damaging to recovery and/or subsequent adaptation necessary to enhance physical performance.

The majority of research examining chronic levels of oxidative stress, inflammatory markers and hormones has studied middle aged and elderly populations. As such, it is unclear whether cigarette smoking in young, otherwise healthy smoking populations elicits any chronic changes in these processes, or whether these are associated with the magnitude of tobacco exposure (number of cigarettes per day and the duration of regular smoking). Recently, however, it has been found that smokers may exhibit differing acute biochemical responses to physical work than non-smokers, evidenced by greater increases in oxidative stress markers following treadmill running in young active smokers (Bloomer, Creasy & Smith 2007). Given the purported roles and interactions between redox balance, endocrine status and chronic inflammation in the maintenance of muscle and bone health, if these processes are altered in smokers in a military training population these may mediate training adaptation and influence injury risk.

The Infantry Training Centre, Catterick (ITC(C)), is the largest British Army training establishment, and houses the training of all line infantry trainees. The parachute regiment and line infantry courses are both administered at ITC(C) and have been shown to have the lowest first time pass rate of trainees in the British Army (40-50%; Carter et al. 2006) and the highest medical discharge rate (Blacker et al. 2005). As such, this training environment provides a large representative sample of British military trainees where novel research can be performed to compare the improvement in performance between smokers and non-smokers in a young, physically active population alongside blood biochemical analysis. Additionally, despite the purported link between cigarette smoking and injury risk,

smoking habits of trainees at ITC(C) have not been examined, and a comprehensive evaluation of injury occurrence has not been completed in this population.

Based on the above background, this work was commissioned and funded by the Army Recruiting and Training Division (Ministry of Defence, UK) to examine a number of novel research questions previously unexamined in a British Army training population. This was to collect information that could potentially inform on policy for smoking and physical fitness in British Army training as well as improving trainee education.

The research described in this thesis will aim to answer the following research questions:

- 1) What is the prevalence of smoking in military trainees at ITC(C), and what is the extent of trainee tobacco exposure?
- 2) What are the resting concentrations of selected markers of oxidative stress, inflammation and hormones of habitual smokers at entry to training, and is there evidence of a dose-response relationship to cigarette consumption in these markers?
- 3) Is physical performance improvement and muscular adaptation impaired in habitual smokers during military training, and do alterations in resting markers of oxidative stress, inflammation and hormones reflect any differences in adaptation in smokers?
- 4) Do habitual smokers exhibit different acute biochemical responses to bouts of military exercise that may indicate greater physical strain compared to non-smokers?
- 5) Does a higher risk of training-related injury exist in habitual smokers at ITC(C) when compared to non-smokers?

CHAPTER 2

Review of Literature

Review of Literature

2.1 Cigarette smoking

2.1.1 Smoking statistics

Habitual cigarette smoking is the greatest preventable cause of chronic illness and premature death worldwide (Fagerström 2002; World Health Organisation 2004), estimated to be responsible for 5.4 million deaths in 2004 (World Health Organisation 2004). In Britain, smoking is currently estimated to cause 18% of deaths (~100,000 per year; Britton 2012), and the total economic cost of health care services for smoking-related illnesses is ~£2.7 billion (Callum, Boyle & Sandford 2011).

The prevalence of smoking in Britain was approximately 20% in 2010, following a slow steady decline from 1998 (Dunstan 2010). Smoking prevalence is, however, highly variable depending on age, marital status and socio-economic status. When socio-economic status is classified by job type, smoking prevalence peaks at 30% in manual working males between the ages of 25-34 years (Robinson & Bugler 2008; Table 2.1). Typically, smoking prevalence is inversely proportional to age, education and socio-economic status. In support of this, the opposite relationship exists to completing successful smoking cessation (Vangeli et al. 2011). Additionally, on average while smoking prevalence is currently similar in males and females, married individuals are less likely to smoke than those who live alone or cohabit with same sex individuals.

The average cigarette consumption rate in Britain is ten cigarettes per day, and, contrary to prevalence, has remained relatively constant for the last two decades (Robinson & Bugler 2008). The assessment of cigarette consumption has also led to research attempting to quantify lifetime tobacco exposure. Current epidemiology research measures this in pack-years, calculated by cigarettes smoked daily divided by 20 (1 pack), multiplied by years smoked.

Limitations exist in smoking epidemiology, however, which may cause both prevalence and exposure to be under- or overestimated. For practical simplicity, research tends to group individuals into those who currently smoke, and those who

do not. This assumes little disparity between never-smokers and former regular smokers, and disregards the considerable variation in cigarette consumption that exists within current smokers. Smoking behaviour is often self-reported, introducing inaccuracies from recollection, and potential error from social bias (Kozlowski 1986; Huerta et al. 2005). For instance, responders tend to round cigarette consumption down to the nearest multiple of ten (Kozlowski 1986), meaning the majority of figures are likely to be underestimates.

Table 2.1. Average percentage smoking prevalence in UK males in 2008 organised by age and socio-economic status. Table adapted from the General Lifestyle Survey, Office for National Statistics (Robinson & Bugler 2008)

Group	Smoking Prevalence (%)	Pooled Average (%)
National Average	21	
Age		
16-19	18	
20-24	29	
25-34	30	
35-49	24	
50-59	23	
Socio-Economic Group		
<i>Managerial and professional</i>		
Large Employers and higher managerial	11	
Higher Professional	12	14
Lower Managerial and professional	16	
<i>Intermediate</i>		
Intermediate	21	
Small Employers	22	22
<i>Routine and Manual</i>		
Lower Supervisory	26	
Semi-Routine	31	30
Routine	33	

2.1.2 Cigarette composition

The constituents of cigarettes have been of considerable interest in tobacco control research, both in the interests of public health (Pryor 1997; Baccarelli et al. 2002; Hecht 2006) and smoking addiction (Jarvis 1987; Vangeli et al. 2011). Unfortunately, research is hindered by the composition of branded cigarettes often kept confidential by the manufacturer. As such, while it is known that cigarettes can be composed of thousands of substances, only a few hundred have been extensively researched (Baker, Pereira da Silva & Smith 2004). Given the complex chemical reactions during combustion, establishing which inhaled products result from which original constituents presents a challenge (Smith & Fischer 2001).

Research has centred on examining substances after combustion in an attempt to describe the composition of inhaled tobacco smoke (Baker et al. 2004; Calafat et al. 2004; Wilson et al. 2008). However, considerable variation exists in the length, filters and rate of combustion of different branded cigarettes, which further alter the composition of tobacco smoke (Hoffmann & Hoffmann 1997). Generally, mainstream cigarette smoke (smoke inhaled directly from a lit cigarette) has been shown to contain ~4700 identified constituents (Hoffmann & Hoffmann 1997; Wilson et al. 2008). Approximately 500 of these are volatile compounds and 3500 are semi- or non-volatile, all existing either as gas or as suspended particulate matter (Hoffmann & Hoffmann 1997; Wilson et al. 2008). It has been observed that a minimum of 60 of these substances are widely established carcinogens (Hecht 2006). Specific research attention has been given to tar and nicotine content of cigarettes. The former has been observed to be a strong predictor of cigarette toxicity and associated lung disorders, and the latter the primary factor in tobacco dependence (Hoffmann & Hoffmann 1997; Fowles, Bates & Noiton 2000).

2.1.3 Smoking and health

It is well established that cigarette smoking adversely affects long term health. Smoking is implicated in the pathology of cardiovascular (He et al. 1999) and pulmonary diseases (He et al. 1999; Birrell et al. 2008; Taylor 2010), metabolic and immunological disorders (Al-Delaimy et al. 2002; Soporì 2002; Birrell et al.

2008), and is thought to be the cause of one third of all cancers (Doll et al. 2005; Robinson & Bugler 2008). There is also considered to be a dose-response relationship, whereby greater cigarette consumption increases risk of developing associated illness (Law et al. 1997). Smoking is also associated with a shorter lifespan. In a longitudinal study detailing the lives of doctors over 50 years, it was observed that smokers, on average, lived 10 years less than non-smokers (Doll et al. 2005), and it was estimated that 50% of smokers died as a result of smoking-induced illness (Doll et al. 1994).

Following inhalation of tobacco smoke, the complex interplay of processes within host defence and the circulation, as well as the composition of the smoke, create a challenge for researchers to establish the pathology of specific illnesses. However, it is generally considered that the inciting mechanisms involve carbon monoxide, reactive oxygen species (ROS), and the obstructive effect of particulate matter. Carbon monoxide, formed from incomplete combustion of particulates, has a higher affinity to haemoglobin than oxygen (Von Burg 1999). Subsequently, the inhalation of carbon monoxide impacts on circulatory oxygen transport (Silverstein 1992) and can cause local tissue hypoxia (Leone et al. 2008). Free radicals produced in cigarette smoke cause local increases in oxidative stress within lung tissue and alveoli (Pryor 1997; Taylor 2010), causing oxidative damage (Nair et al. 1996). Both oxidative damage to tissue and the presence of particulate matter stimulate a local influx of neutrophils and phagocytic macrophages (Tidball 2005). This is supported by acute increases in pro-inflammatory cytokines observed immediately after smoke inhalation (Van der Vaart et al. 2004). While it will be discussed in more detail later, it is observed that smoking has a similar impact within the circulation (Helmersson et al. 2005; Basu et al. 2009), which is associated with the pathogenesis of many long-term health conditions.

2.1.3.1 Smoking and cardiovascular disease risk

Smoking is associated with higher risk of cardiovascular disease, myocardial infarction and atherosclerosis (He et al. 1999; Smith & Fischer 2001; Bazzano et al. 2003). Long-term smokers often exhibit chronic low-grade systemic inflammation (Levitzky et al. 2008; Sunyer et al. 2009) and elevated blood pressure (Al-Safi

2005). Alongside carbon monoxide-induced hypoxia of cardiac muscle in animal models (Penney & Formolo 1993; Loennechen et al. 1999; Sørhaug et al. 2006), these factors are possible mediators in the increased risk of myocardial infarction. It is likely that the elevated presence of fibrinogen (Sunyer et al. 2009) and endothelial cell dysfunction (Celermajer et al. 1993; Poredos, Orehek & Tratnik 1999; Tanriverdi et al. 2006) in smokers accelerates the formation of atherosclerotic plaques (Celermajer et al. 1994; Zeiher et al. 1995).

2.1.3.2 Smoking and pulmonary health

There is some ambiguity, however, as to whether smoking adversely effects lung capacity, or lung structure and function (Gold et al. 1996; Anthonisen, Connett & Murray 2002). Several studies have found that, when compared to non-smokers, no differences in lung capacity are evident in smokers (McCarthy, Craig & Cherniack 1976), but parameters indicating the rate of expiration are reduced (De & Tripathi 1988). Increased populations of mast cells and abnormalities in lung compartments have been implicated in altered airway structure and function in smokers (Ekberg-Jansson et al. 2005; Just et al. 2005). The accumulation of particulate matter within the lungs and the damage to lung tissue from habitual smoking are thought to be functional in the development of chronic obstructive pulmonary disease (Gualano et al. 2008; Taylor 2010), a condition where the immune response in the lung tissue becomes impaired and airways become chronically inflamed.

2.1.3.3 Other health effects associated with smoking

Smoking alters immune function, subsequently increasing risk of immunological disorders (Sopori 2002; Birrell et al. 2008). Macrophages isolated from the bronchoalveolar lavage of smokers have been shown to have impaired function and induce different cytokine responses in comparison to those in non-smokers (Mio et al. 1997; Birrell et al. 2008). Wound healing is also impaired in

smokers, possibly mediated by increased inflammation, reduced blood flow and tissue hypoxia (Sherwin & Gastwirth 1990; Silverstein 1992).

Typically, between adolescence and young adulthood, smokers have lower body fat than non-smokers despite similar caloric intake (Klesges et al. 1990). This also manifests in weight gain upon smoking cessation (Klesges et al. 1992). This is understood to be, in part, owing to the effects of nicotine on appetite suppression, altered substrate utilisation and increased nervous activity (Seeley & Sandoval 2011; Martínez de Morentin et al. 2012). As such, this disparity is not from health-promoting behaviour, as smokers typically exhibit lower physical activity (Larsson & Orlander 1984) and less healthy diets (Beser et al. 1995; Palaniappan et al. 2001; de Castro & Taylor 2008). In agreement, evidence has shown smokers to have lower dietary intake of macronutrients, reduced levels of antioxidants and be more at risk of being nutrient deficient compared to non-smokers (Reilly et al. 1996; Dyer et al. 2003; de Castro & Taylor 2008).

2.1.4 Smoking and physical fitness

Epidemiological evidence has shown that habitual smoking is associated with lower cardiorespiratory fitness (Bernaards et al. 2003; Kobayashi et al. 2004). However, it is difficult to disregard that this association may exist from smokers typically exhibiting lower physical activity and participation in sports (Larsson & Orlander 1984; Larson et al. 2007). Ideally then, to accurately examine the impact of smoking on long term fitness, studies should assess individuals of similar ages, with similar physical activity levels and body composition, or take appropriate steps to control for these factors. The acute effect of smoking on physical performance in exercise tasks has also been examined, generally observing a negative impact, and will be discussed later in this section. Consequently, many assertions have been formulated from the assumption that the acute effects of smoking may be cumulative and chronically impact exercise performance.

2.1.4.1 Chronic effects on physical performance associated with smoking

Surprisingly few studies have examined the difference in physical fitness parameters between habitual smokers and non-smokers. A longitudinal study completed by Bernaards et al. (2003) observed, between the ages of 13 and 36, maximal oxygen uptake ($\dot{V}O_{2\max}$) and maximum heart rate were negatively correlated with cigarette consumption both with and without correction for physical activity and body mass. Kobayashi et al. (2004) observed that $\dot{V}O_{2\max}$ values made relative to body mass were significantly lower in smokers, likely owing to greater fat mass in the smoking individuals. Potential mechanisms for the negative influence of chronic smoking on cardiorespiratory fitness are typically altered airway size (McCarthy et al. 1976), impaired pulmonary muscle function (Barreiro et al. 2010), less efficient respiratory exchange (Gläser et al. 2011), reduced oxygen transport to muscles (Rönnemaa et al. 1999) and higher blood pressure (Al-Safi 2005).

Other physical fitness parameters have also been shown to be lower in smokers when compared to non-smokers. A study examining lower back pain showed reduced force production in habitual smoking individuals (Al-Obaidi et al. 2004). Trends have also been elucidated for lower bench press performance and flexibility in smoking versus non-smoking police officers (Boyce et al. 2006). Conversely, in smokers and non-smokers matched for age and physical activity, two studies have shown that while maximal strength and contractile speed were similar between groups, it was fatigue resistance that was reduced in habitual smokers (Morse et al. 2007; Wüst et al. 2008). Lower muscular oxidative capacity and stimulation frequency of muscle in smokers were theorised as possible reasons for these findings (Larsson & Orlander 1984; Morse et al. 2007; Wüst et al. 2008).

Interestingly, studies in long term smoke exposure in rats suggested differing muscle morphology, specifically lower muscle cross sectional area alongside fewer type I oxidative fibres compared to controls (Larsson et al. 1988). Although this effect was only evident in hypertensive animals, in humans similar findings have previously been observed in smoking and non-smoking twins (Larsson & Orlander 1984). Research to determine the underlying mechanisms for altered muscle morphology has remained inconclusive. However, it is considered

that the accumulation of the acute effects of smoking a cigarette on subsequent exercise performance may contain the mechanisms for the discussed findings.

2.1.4.2 Acute effect of smoking on physical performance

Previously, specific attention has been given to the acute effects of cigarette smoking or smoke exposure on exercise performance. It is known that the act of smoking a cigarette increases resting heart rate and blood pressure (Rotstein et al. 1991). Similarly, in response to subsequent exercise, habitual smokers exhibit increased heart rate for a given work load and delayed heart rate response to exercise (Sidney et al. 1993). Research has shown an immediate negative effect of smoke exposure on attainment of $\dot{V}O_{2MAX}$ and anaerobic threshold (Hirsch et al. 1985). However, this study administered unrealistic smoke exposure, with individuals smoking three cigarettes an hour for five hours prior to exercise. More recently, studies have observed smoking immediately prior to exercise reduces time to exhaustion (Mendonca, Pereira & Fernhall 2011) possibly mediated by reduced power output or maximum attainable heart rate (Flouris et al. 2010; Mendonca et al. 2011). Additionally, Morse et al. (2008) reported that inhalation of carbon monoxide decreased muscular time to fatigue in electrically induced muscle contractions.

Interestingly, the effect of administration of nicotine in non-smoking cyclists was a significant 17% improvement in time to exhaustion (Mündel & Jones 2006). Nicotine has well established effects on alertness and cognitive performance, but similar levels of perceived exertion, ventilation and heart rate response were observed between trials. It is reasonable to conclude, then, that nicotine may have an advantageous effect on non-smokers where nicotine tolerance is low, and neither the acute or chronic effects of cigarette smoking are present.

2.2 Military training

Basic military training has the aim of producing and identifying effective soldiers, physically and mentally able to perform required occupational tasks. Although training will vary between countries and their internal branches of armed forces, the overarching aims and structure are typically relatively similar. Military training consists of a mixture of physical, drill and skill-based training alongside classroom teaching. Within this structure there are also short periods (~3-15 days) of intense field training exercises. Some nations have compulsory enlistment, where a set term of military service must be completed by every male of a particular age preceded by basic training. For others, joining a military career is voluntary, and following training there is a minimum duration of service. For basic training of soldiers, as opposed to higher ranking officers, most countries employ between 8 and 14 weeks for basic training (Booth et al. 2006; Tanskanen, Uusitalo, et al. 2011).

2.2.1 British Army training

In the British Army, individuals can enter into officer training or “other ranks” below that of officer. Officer training is a set training duration at Sandhurst, and is followed by further training for a specific role. Entry for “other ranks” will contain a basic training course, where different training regiments train for various specialties. The British Army take on standard entrants aged between 17 and 33 years old.

Standard entrants wishing to join the British infantry complete a 26-week Combat Infantryman’s Course (CIC), which consists of 14 weeks of standardised basic infantry training, and a further 12 weeks of training specific to their regiment. The Infantry Training Centre, Catterick (ITC(C)) is the largest training establishment in the British Army, and is responsible for the training of all divisions of line infantry, and Guard and Parachute regiments. Every two weeks, new intakes of up to three platoons (~50 trainees per platoon) of line infantry recruits are initiated. As such, ITC(C) sees in excess of 3000 recruits per annum. Apart from the

exceptionally arduous training of the Parachute Regiment, CIC at ITC(C) has the lowest first-time pass rate into the British Military at 40-50% (Carter et al. 2006).

Recruits wishing to join other services in the British Army attend an Army Training Regiment (ATR) establishment specific to their choice of prospective trade. As each member of the armed forces must fundamentally be able to function in the infantry, all recruits first complete the standardised template for basic infantry training, similar to that of CIC, followed by training specific to their desired service.

2.2.2 British Army physical training

Military basic training is composed of a large variety of physical fitness training. Arguably the most representative physical task of infantry personnel is to carry heavy burdens continuously over long distances. As such, the main fitness objectives of basic training are to increase aerobic capacity and performance in endurance exercise while carrying load. Military training contains wide varieties of runs, circuit training and loaded marches where mass carried and distance covered progress over training duration. Research into physical demands of CIC has shown that energy demand increases substantially to a peak over the first six weeks of training (Carter et al. 2006). This suggests that the development of the required physical fitness is not expected until near the end of training. Rather, the first six weeks are designed to progress trainees to fitness goals.

The physical fitness of trainees is monitored during physical training sessions and in a number of testing environments. The Army Physical Fitness Test (PFT) consists of a timed best-effort 2.4 km run, and the completion of press ups and sit ups when allowed 2 minutes for each exercise. Completed at the start, middle and end of military training, this assesses progression through the course. Performance in the basic combat fitness test, a loaded march scenario, is also monitored at specific points during training as an indicator of readiness for the occupational demands of battle. Both tests have pass-criteria highlighting individuals who may need further training or not be up to the physical standards required. It has been shown in previous work that performance in equipment carry and marching tasks are strongly correlated with various physical performance

measures such as static lift strength and run times (Rayson, Holliman & Belyavin 2000). Additionally, research has found that owing to the load bearing nature of many military tasks, anthropometric measures such as lean body mass are strong predictors of occupational performance (Vanderburgh 2008). Military training, therefore, has several different physical fitness goals for trainees to attain in preparation for military service.

It should be noted that the majority of physical training in military populations is completed in groups or as a platoon. This means trainees of very different fitness levels will often run long distances as a group at a specific predetermined intensity. Although this may hinder fitter individuals from progressing to higher fitness goals, it is conducive to the majority of recruits reaching a particular fitness standard. Alongside physical training, field exercise has components of physical effort required. Field exercise typically involves a number of days in an outdoor “wild” environment outside the training camp itself, testing navigation and survival skills.

2.2.3 Military field exercise

Field training is designed to test a variety of physical and survival skills over several days. As such, it is considered the most comparable environment to conditions that may be experienced during war deployment. Therefore, a volume of research has examined several aspects of field training, including hormone responses and energy balance. Training ranging from 8 to 15 days, has shown an average of ~6 hours of non-continuous sleep per day, typically disrupted to complete military tasks (Nindl et al. 2003; Kyröläinen et al. 2008; Tyyskä et al. 2010). Alongside this, the frequency of marching and low caloric intake can produce substantial energy deficit (Kyröläinen et al. 2008). It is suggested that extended periods of training of this nature can have profound effects on increasing levels of stress and altering hormone control (Booth et al. 2006; Nindl, Barnes, et al. 2007).

2.2.4 Injury in military training

Although the positive health benefits of physical activity are well established, the commencement of exercise in any population carries with it a higher risk of musculoskeletal injury. Owing to the arduous and often unaccustomed nature of physical training to new military trainees, there is a high incidence of training-related injury (Knapik et al. 1993; Carter et al. 2006). The potential loss of service time, long-term rehabilitation and possibility of re-injury associated with an injury is of great cost both economically to the military, and to the individual trainee. Therefore, the examination and prevention of injuries during training is of considerable interest to military organisations. In this regard, research describing injury incidence (Kaufman, Brodine & Shaffer 2000; Knapik, Canham-Chervak, et al. 2001), identifying risk factors for training injuries (Altarac et al. 2000; Knapik, Sharp, et al. 2001; Blacker et al. 2008) and interventions for the possible prevention of injury (Knapik et al. 2004; Bullock et al. 2010) have been conducted within military populations.

Research into injury incidence is abundant in United States (US) and Scandinavian military populations but has reported highly variable results with rates as high as 51% in US infantries (Knapik et al. 1993), 40% in US marines (Almeida et al. 1999), 24% in Norwegian basic training (Heir & Eide 1997) and 32% in Royal Norwegian Navy personnel (Morken, Magerøy & Moen 2007). Studies in British training establishments have reported the prevalence as high as 46 and 49% of recruits sustaining an injury (Etherington & Owen 2002; Greeves 2002). In a large-scale study of the kind not previously undertaken on British Military trainees, Blacker et al. (2005) investigated the rates of training-related injuries referred to remedial instructors in several different British training establishments, where values ranged from 1.4% to 26.5% over training courses and locations. It is evident that differences in training content and environment that are inherent with different training locations alter injury risk, even within the same military organisation. There has not been a comprehensive injury incidence research study completed at ITC(C).

The proportion of medical discharge from recruit training owing to training-related injury has been reported in several locations. The percentage of medical

discharge in male and female junior entrants at ATR Bassingbourn was 1.4% and 12.8%, respectively, and 1.6% in standard entrants at ATR Pirbright (Etherington & Owen 2002; Blacker et al. 2005). Injury incidence in junior and standard recruits across all British training establishments except ITC(C) showed an average of 18% of recruits who sustained an injury were later medically discharged as a result (Greeves 2006). When corrected for number of days in training, ITC(C) has the highest medical discharge rate among recruits in the British military (Blacker et al. 2005). As such, it is evident that research to better understand the nature of training-related injuries may aid strategies to improve first-time pass out rate.

The types of injuries most commonly reported in military populations are musculoskeletal overuse injuries predominantly in the knee and lower leg (Kaufman et al. 2000). Predominantly diagnosed injuries in a review containing several military populations were lower back pain, muscle strains, ankle sprains, shin splints, lower-leg stress fractures and overuse knee injuries such as patella-femoral syndrome, patellar tendinitis and ilio-tibial band syndrome (Jones et al. 1993; Jones & Knapik 1999; Kaufman et al. 2000). In British Army recruit training, injuries to the back, foot and knee were the most common comprising 50% of reported injuries (Greeves 2006). In ATR Pirbright lower limb injuries accounted for 82.9% of all training injuries (Etherington & Owen 2002). Injuries of this nature are highly representative of military training, which often involves repetitive exhaustive load-bearing exercise that largely affect the lower-limb and supporting musculature.

Frequency of injury type is highly variable between populations, with stress fractures highest (13.4%) in Naval Special Warfare Training (Kaufman et al. 2000), ankle sprains (6.2%) highest in US marine recruits (Almeida et al. 1999) and lower back pain (7.8%) highest in Army infantry basic training (Jones et al. 1993). Like absolute injury prevalence, this suggests injury type is also highly dependent on the type of training performed by different military organisations. However, it should be noted that in all injury incidence research, some variability between studies can be, in part, explained by differences in the methodology behind defining and recording injuries and detailing anatomical locations.

The time-loss from injury is also responsible for the impact of injury on the military. In military populations the time-loss caused by injury has been shown to

be often substantially higher than from illness (Jones & Knapik 1999). Knapik et al. (1993) documented limited-duty days (days when unable to fully perform on duty) from injury for several injury types, reporting fractures most severe (103.2 days lost/injury) followed by sprains (16.7 days lost/injury). Training loss of this nature has serious negative implications towards physical performance, skills training and, if not adequately rehabilitated, risk of re-injury.

Risk factors appear in two defined groups, intrinsic (within the individual) and extrinsic (external, environmental factors such as equipment or clothing). The most commonly reported risk factors for injury in military populations are low aerobic fitness, gender and cigarette smoking. Observed military risk factors from the literature are summarised in Table 2.2. Military researchers have identified several risk factors for injury including intrinsic factors: low aerobic fitness, low levels of previous physical activity (Kaufman et al. 2000; Knapik, Sharp, et al. 2001); previous injury to the same site (Schneider, Bigelow & Amoroso 2000), female gender (Greeves 2006), age, ethnicity and some biomechanical factors (Kaufman et al. 2000); and extrinsic factors such as cigarette smoking (Etherington & Owen 2002), footwear type and training location (Blacker et al. 2008).

2.3 Smoking in the military

This section will highlight some of the issues surrounding smoking within military populations. Given the numerous effects on health and physical fitness discussed above, there are many potential avenues for adverse effects of habitual smoking on operational performance in the military. Previous research has attempted to examine the variation in smoking prevalence both in different branches of military populations and in different nations; the attitudes surrounding tobacco use within the military; and the increased risk of training-related injury associated with smoking. Despite comprehensive coverage of many influences of smoking in current research, few studies have looked at the effect of habitual smoking on physical performance or development of fitness in military training.

Table 2.2. Risk factors for injury in various military populations.

Risk Factor	Supporting Literature
Lower aerobic fitness ($\dot{V}O_2$ max or military performance variables)	$\dot{V}O_2$: Knapik et al. (2001); 3km run time: Reynolds (1999), Heir & Eide (1997); 2.4km run time: Blacker et al.(2005), Blacker et al.(2008), Greeves et al. (2007), Jones et al. (1993)
Lower previous physical activity	Knapik et al. (2001), Heir & Eide (1997), Jones et al. (2000)
Previous injury	Schneider (2000), Etherington & Owen (2002)
Cigarette smoking	Altarac (2000), Etherington & Owen (2002), Knapik (2001), Reynolds (1999)
Age	Low age bracket: Reynolds (1999), Knapik (2001), Blacker et al. (2005). Higher age bracket: Jones et al. (1993), (for stress fracture), Lappe et al. (2001)
Non-white Ethnicity	Blacker et al. (2008), Schneider (2000)
Female gender	Greeves et al. (2006)
Biomechanical factors	Kaufman et al. (2000)
High and low flexibility	(In men) Knapik et al. (2001)
Low body Mass/BMI	Blacker et al. (2008)

2.3.1 Smoking prevalence in the military

While military populations are required to maintain certain levels of health and physical fitness, smoking prevalence is higher than in the general population (Hooper et al. 2008; Dunstan 2010). The smoking prevalence of key studies examining military populations are summarised in Table 2.3, organised by trainees and those within active service. It would appear that the average smoking prevalence in the military from current research is ~30%. Considerable variation exists however, between trainees and individuals in active service, as well as by

country and the military organisation being examined. Furthermore, where a set term of military service is compulsory in specific countries, or when smoking is banned during some basic military training courses, prevalence can be confounded.

The largest variation in results within current research appears to be nationality, where conscripts from Scandinavian countries have reported 48-51% prevalence, compared to substantially lower values in US and UK branches of armed forces. However, given that both Norway and Switzerland exercise compulsory enlistment for a fixed term, these values could be an indication of national average for young adult males. In the British military, reported values range from one study reporting 45% in infantry soldiers (Reynolds et al. 1999), to 13% in Royal Marines (Munnoch & Bridger 2007). Similar variation is reported in US branches of the military. Perhaps most profound, however, is different smoking prevalence within the same, or similar, populations. Both Chisick et al. (1998) and Klesges et al. (2001) examined trainees of all branches of the US Military but found substantially different results, with the former observing a range of 6-27%, and the latter 29-45%. While some of this variation can be attributed to training location and smoking restrictions during training, it is clear that using robust methods when measuring smoking prevalence is critical to maintaining validity.

Smoking prevalence in trainees is similar to that of active-duty personnel. In four studies, trainees during basic training have reported smoking prevalence as low as 6% and 22% in the US Air force (Chisick, Poindexter & York 1998; Sherrill-Mittleman et al. 2009), 13% in Royal Marines (Munnoch & Bridger 2007) and 24% in the Norwegian Army (Heir & Eide 1997). In contrast, however, Altarac et al. (2000) and Klesges et al. (2001) observed higher smoking prevalence in US Army trainees than values reported in service (Chisick et al. 1998; Rae Olmsted et al. 2011). As smoking is often negatively associated with age, it would seem reasonable that the recruit populations would have a greater number of smokers than their active duty counterparts. This may be, in part, explained by a number of factors concerning attitude to smoking within the military, discussed later in this section.

Table 2.3. Average prevalence and number of smokers reported in key papers organised by trainee and active service populations

Study (Author/Year)	Population	Total Sample Size (N)	Smoking Prevalence (%)
Trainees			
(Altarac et al. 2000)	US Army	1087	35
(Munnich & Bridger 2007)	UK Royal Marines	1115	13
(Chisick et al. 1998)	US Army	535	16
	US Air Force	751	6
	US Marines	716	19
	US Navy	709	27
(Heir & Eide 1997)	Norwegian Infantry	480	51
(Miedinger et al. 2006)	Swiss Army	2604	48
(Greeves 2006)	UK Army	1854	37
(Klesges et al. 2001)	US Army	N/A	41*
	US Air Force	29044	29
	US Marines	N/A	45*
	US Navy	N/A	39*
(Sherrill-Mittleman et al. 2009)	US Air Force	35986	22
Active Service			
(Chisick et al. 1998)	US Army	2002	34
	US Air Force	1261	26
	US Marines	243	40
	US Navy	1097	34
(Hooper et al. 2008)	UK Armed Forces	1382	28
(Reynolds et al. 1999)	UK Light Infantry	194	45
(Rae Olmsted et al. 2011)	US Army	5927	30
	US Air Force	7009	22
	US Marines	5117	29
	US Navy	6637	29
(Fear et al. 2010) (Data from (Hotopf et al. 2006))	UK Army		
	Royal Navy/Marines	10272	30
	Royal Air Force		
(Schei & Sjøgaard 1994)	Norwegian Military	2112	51

*Values estimated from the assumption of an identical ratio of drop out to data collected from US Air Force

2.3.2 Smoking exposure in the military

The predominance of studies examining military populations have sought to measure prevalence rather than the total exposure of individuals to tobacco. These studies would typically categorise those who are smokers and non-smokers from enlistment data or in self report questionnaires at the time of the study. Studies that have examined the number of cigarettes smoked per day in active service, have found the average to fall between 10 and 20 (Boos & Croft 2004; Haddock et al. 2007; Fear et al. 2010). At present little information is known about the smoking characteristics of individuals during or prior to joining military training. Without accurately attaining habitual smoking duration it is not possible to estimate lifetime tobacco exposure in these populations. Gathering this information and demonstrating the severity of smoking exposure may aid studies in estimating the potential health risks of habitual smokers during and following military service.

2.3.3 Attitudes to smoking in the military

The incidence of smoking in the military may be explained by inherent psychological and social influences. Cigarette smoking can act as stress relief (Fidler & West 2009) and a communal bonding activity (Nelson, Pederson & Lewis 2009), that may indirectly lend support in stressful situations, often encountered in military service. This is reinforced by evidence that cigarette consumption increases with war deployment (Boos & Croft 2004; Smith et al. 2008). Similarly, it is likely that the long-term health effects of smoking are not of major concern to those involved in high-risk occupations. Furthermore, smokers are often associated with greater risk taking behaviour (Zuckerman & Kuhlman 2000), a personality trait that may be conducive to military service, and therefore prevalent in these populations. These factors are not hindered by the ease of access to cheap tobacco in military environments (Nelson et al. 2009).

The above factors, and studies that have shown the disparity between trainee and active duty smoking prevalence, have led some researchers to believe that the military training environment may indirectly promote tobacco use (Nelson et al. 2009). Indeed, research has shown that products have been marketed specifically to

military populations by tobacco companies (Joseph, Muggli, et al. 2005), and there exist tobacco-funded recreation and welfare programs (Arvey & Malone 2008). It is actually unclear how military training environments affect smoking status, as very few studies have measured smoking status prior to- and following- military training. One study observed that during military training the proportion of those who take up smoking is higher than those who quit (Schei & Sjøgaard 1994). Additionally, a tendency for non-smokers to begin smoking upon joining military training has also been shown (Ebbert et al. 2006). On the contrary though, it would seem possible that physical fitness and health requirements associated with military training would cause either smoking prevalence to reduce or smoking cessation to occur prior to entry.

An association between smoking incidence and both military rank and socioeconomic status has been observed (Fear et al. 2010). It is clear that environmental factors prior to training such as education and lifestyle during upbringing markedly affect the likelihood of habitual smoking in later life. Research findings have led military organisations to implement education and cessation programs to clarify the risks of smoking to personnel, and the benefits of cessation or abstinence from smoking (Arvey & Malone 2008). However, the success of these programs is not clear. Although these measures are positive, it seems that further work into the severity of the adverse influence of smoking and alterations to tobacco control measures in military environments would be necessary to cause any drastic alterations in attitudes to smoking in these populations.

2.3.4 Smoking and the risk of training-related injury

Cigarette smoking is the most widely reported independent risk factor for training-related injury in military populations (Reynolds et al. 1999; Altarac et al. 2000; Knapik, Sharp, et al. 2001; Etherington & Owen 2002). It has been reported that habitual cigarette smokers can have 1.7 times the relative risk of training-related injury than non-smokers (Munnoch & Bridger 2007). There has also been observed a dose-response association, where risk of injury increases with cigarette consumption (Knapik, Sharp, et al. 2001).

Presently, it is unclear what underlying mechanisms there are, if any, for the association between higher injury risk and smoking. However, given the associated effects on health, physical fitness and physiological processes from smoking, any mechanisms for the effect on injury susceptibility are likely to be numerous and complex. Mechanisms for the potential influence of smoking on injury risk in habitual smokers have been postulated. In military populations, reduced physical fitness (Kobayashi et al. 2004) and lifetime physical activity (Conway & Cronan 1992), both concomitant with smoking, may be particularly functional in establishing a higher risk of injury. It has been theorised that the group nature of physical training in the military means less fit individuals may be trained far in excess of their ability. In this way, having reduced physical fitness may increase likelihood of injury from training (Knapik, Sharp, et al. 2001; Knapik et al. 2004).

Additionally, recovery from training could be impaired in smokers. If recovery is impaired, non-smokers will be in a relatively better state for the completion of progressive exercise training, and have a lesser risk of injury. The typically lower dietary intake (Klesges et al. 1990), impaired immune function (Arcavi & Benowitz 2004) and slower wound recovery (Sherwin & Gastwirth 1990) in smokers suggest recovery from exercise or injury could be lessened. Recovery may also be affected by smoking-induced alterations in oxidative and inflammatory processes (Cross et al. 1998; van der Vaart et al. 2004), and will be discussed in further detail later.

Smoking has a profound adverse effect on bone health and metabolism (Wong, Christie & Wark 2007). Whilst having a direct effect on the risk of stress fractures, this may also have structural implications on soft tissue and supporting musculature. In terms of injury risk, this would be particularly detrimental in repeated bouts of continuous exercise, and subsequently harmful in military training. Lastly, greater risk-taking behaviour (Zuckerman & Kuhlman 2000) observed in smokers may increase the likelihood of inciting events.

2.3.5 Smoking and fitness in military training

The effect of smoking on physical fitness in military populations has rarely been examined. Where physical fitness is typically lower in smokers, it is

challenging to disassociate this from lower physical activity in smokers. In several studies, physical fitness has been shown to be lower in smokers in military populations (Zadoo, Fengler & Catterson 1993; Ward et al. 2003; Haddock et al. 2007). Haddock et al. (2007) showed that smoking status was a strong predictor for poorer performance in a number of physical fitness domains at entry to training. Indeed, smokers have been linked to having poorer training outcomes and poorer run performance in a Scandinavian military population (Marti et al. 1988). The development of muscular fatigue is more rapid in smokers (Morse et al. 2007; Wüst et al. 2008) and, alongside this, reduced force production in back muscles (Al-Obaidi et al. 2004) may affect carrying tasks. This is particularly important as both carrying and back extension strength are deemed robust indicators of effective military occupational performance (Rayson et al. 2000). Given the potential effects on physical fitness, smoking may have some influence on how well trainees perform in training. However, while completing a standardised training programme with comparable baseline fitness, it has not been examined whether smokers' change in fitness substantially differs from non-smokers.

It is unclear, therefore, whether smoking has an effect on the development of physical fitness in a physically active population. One study examining a British Army officer training population over six months observed significantly greater improvements in performance of strength and endurance tests in non-smokers than smokers (Hoad & Clay 1992). Despite this, no research has been completed to further test this hypothesis.

2.4 Physiological effects associated with smoking

As discussed, research examining the development of physical fitness in habitual smokers is sparse, and it is unknown whether differing adaptive responses to exercise training exist in comparison to non-smokers. Given the high incidence of smoking in military training, and the already well-established effect on injury risk, further understanding would be particularly valuable. In this section, a number of physiological processes that are affected by smoking will be introduced and

discussed. These mechanisms centre around the chronic effect of smoking, highlighting the possible physiological state of a long-term habitual smoker. How these processes might serve as mechanisms for mediating the adaptive response to exercise training will be discussed in section 2.5. Figure 2.1 summarises these proposed pathways.

For the coming sections of this literature review and for the purpose of this thesis a number of terms will be used to aid the description of how smoking or training may interact with selected biochemical markers. The term “physiological state” or “physiological status” will refer to the resting levels of the selected oxidative stress, inflammatory and endocrine markers analysed in this research, which indicates the chronic balance of these markers in the examined group. As such, “adaptation” of a oxidative stress, inflammation or endocrine markers will refer to a change in the resting concentration between distal time-points such as between weeks of training. Conversely, the term “biochemical response” will refer to acute, or short-term, changes in the selected biochemical markers in response to a stimulus, such as a bout of exercise, with the implication that markers will return to pre-stimulus level relatively transiently.

2.4.1 Oxidative stress

Classically, oxidative stress is defined as an imbalance of the cellular environment caused by the inability of a biological system to detoxify ROS, or reduce subsequent oxidative damage, at the rate of production. Reactive oxidants, or oxygen radicals, are oxygen-containing molecules with unpaired electrons, which are subsequently chemically volatile. Several processes stimulate the production of oxidants. Often, these are necessary pathways where oxidants serve as intermediates or by-products. Normal cells will always tend towards maintaining a reducing environment, which is the addition of electrons, and a decrease in oxidation number. This state of reduction is maintained by closely regulated enzymes as part of antioxidant defence, and requires a constant influx of metabolic energy (Comporti et al. 2008). ROS then oxidise surrounding molecules in a perpetual chain, either until another oxidant is reached or the reaction is neutralised by antioxidant defence. When the biological system cannot control the

accumulation of ROS the cellular environment shifts away from reduction and towards oxidation, resulting in oxidative stress. This imbalance, and the presence of high levels of ROS, can oxidise lipids potentially causing damage to DNA, and structural and functional components of the cell.

However, with growing understanding, the functional significance of the cellular environment of redox-sensitive cells suggests further complexity, and the definition of the term “oxidative stress” may continue evolving (Powers, Smuder, et al. 2010). Advances in redox biology have shown that the redox balance within the cell has multiple implications for cell signalling, and potentiating transcription factors for mediating future oxidative environments (Powers & Jackson 2008). This has suggested that short-term acute imbalances in oxidation and reduction can be beneficial (Powers, Duarte, et al. 2010), while the persistent shift of cellular environments in favour of oxidation is deleterious. For instance, long-term elevations of oxidative stress are implicated in the ageing process (Harman 1956; Ashok & Ali 1999; Biesalski 2002) and the pathology of many chronic diseases (Lambeth 2007; Valko et al. 2007). The toxicity of oxidative by-products and the modification of lipoproteins from oxidative damage influence atherogenesis, and endothelial cell and macrophage dysfunction (Witztum 1993; Cross et al. 1998; Young & McEneny 2001).

The physiological quantification of oxidative stress has been made possible by advancements in the measurement of by-products from lipid peroxidation, metabolites from oxidation reactions, redox-sensitive molecules and the presence of antioxidant enzymes (Morrow 2005; Powers, Smuder, et al. 2010). Additionally, in animal models and humans, it is also possible to measure the effectiveness of antioxidant defence, known as total antioxidant capacity (TAC), with measurement methods reviewed here (Young 2001). It is understood that measuring multiple markers of oxidation and TAC is the most rigorous method for establishing whether oxidative stress is present, by observing the overall redox status of the tissue (Powers, Smuder, et al. 2010). Research has subsequently examined both acute and chronic levels of oxidative stress, TAC, and dietary antioxidant supplementation.

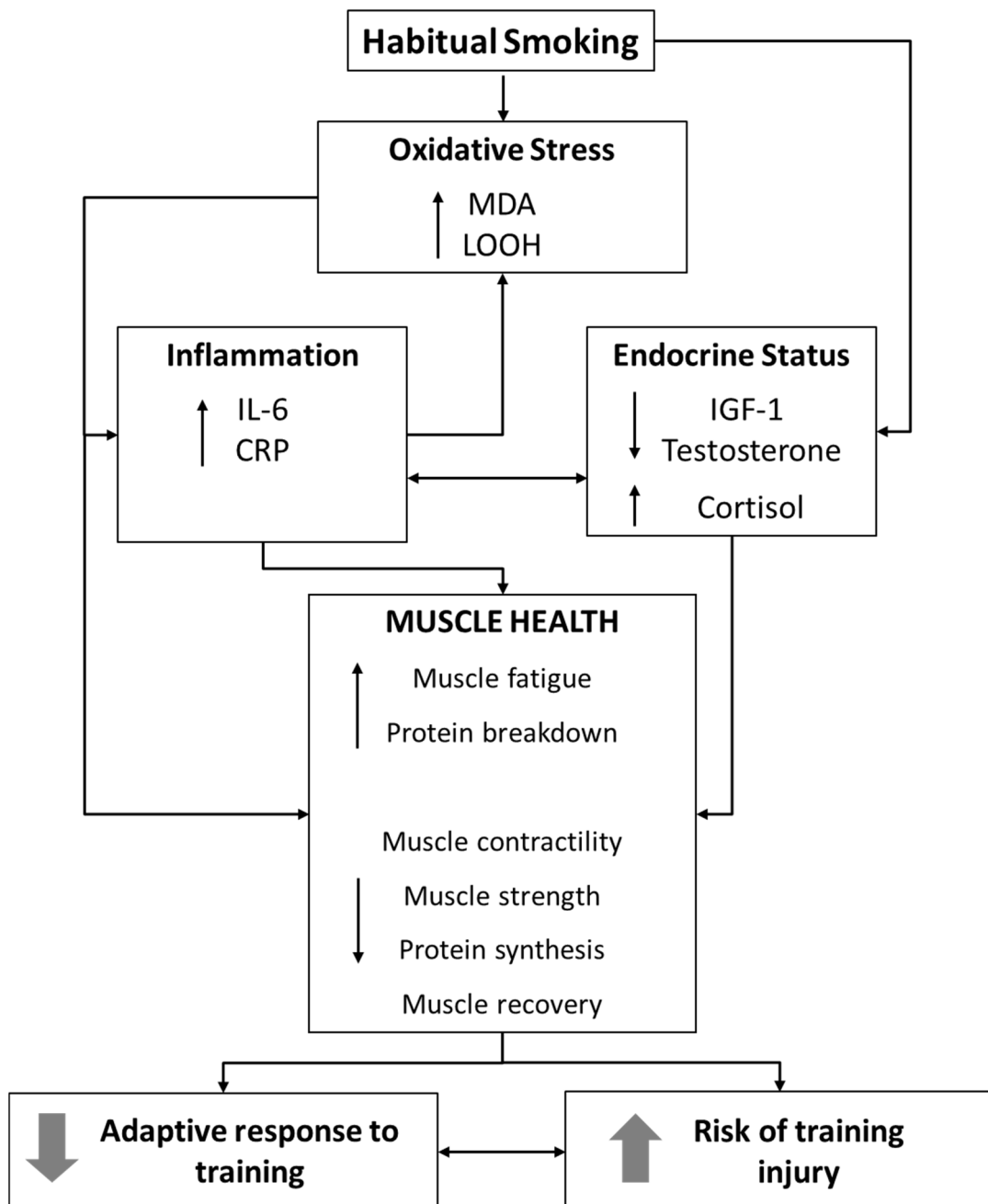


Fig 2.1. A schematic overview of the proposed effects of smoking on markers of oxidative stress, inflammation and endocrine status, and the potential subsequent influences on the mediation of muscle health during exercise training

2.4.1.1 Markers of oxidative stress

Direct quantitation of the magnitude of oxidative stress within a biological system presents many challenges to researchers. Reliable markers of oxidative stress must be unique to the specific process examined, and subsequently respond to imbalances in redox status, and be chemically stable with sufficient half-life to remain detectable (Powers, Smuder, et al. 2010). Typically, substantial production of ROS induces inter- and extra- cellular degradation of lipids through peroxidation. Oxidative damage can also occur to cellular proteins and DNA. As such, the techniques currently used in research to quantify oxidative stress in a biological system are markers of this oxidative damage. Researchers must therefore decide which techniques and markers are suitable for their research question. Detailed commentary of markers of DNA damage, lipid and protein peroxidation is beyond the scope of this literature review, but several of the more commonly used markers of lipid peroxidation will be discussed.

Currently, the recommended “gold-standard” for measurement of oxidative stress is F₂-isoprostanes (Powers, Smuder, et al. 2010). Isoprostanes are prostaglandin-like compounds that are produced as a by-product of oxidation of polyunsaturated fatty acids. F₂-isoprostanes are produced from peroxidation of arachidonic acid in a process solely catalysed by endogenous free radicals, making them a reliable *in vivo* indicator of oxidative damage. Additionally, compared to other markers the relative stability and longer half-life of F₂-isoprostanes and the ability to detect levels in both plasma and urine mean it is particularly appropriate for human research (Morrow et al. 1995). Isoprostanes can be measured using a number of methods (including enzyme-linked immune-sorbent assay (ELISA)) but have the advantage of being detectable by gas chromatography/mass spectroscopy (GC-MS) which, though costly, is a highly reliable measurement technique. Assays are available for the measurement of F₂-isoprostanes but have been reported to have variable results and further work is needed to reach the precision of other measurement techniques.

Malondialdehyde (MDA) is the principal product of peroxidation of polyunsaturated fatty acids, and one of the most widely examined markers of lipid peroxidation (Del Rio, Stewart & Pellegrini 2005). As a relatively stable molecule,

research has predominantly examined chronic levels of MDA in disease pathogenesis. MDA has been shown to increase during infection, and have diurnal variation that correlates with white blood cell count (Akbulut et al. 2003). Similarly, MDA correlates with biomarkers of T-cell activation, concurrent with cancer generation and atherosclerosis (Kolanjiappan, Manoharan & Kayalvizhi 2002; Tamer et al. 2002; Akbulut et al. 2003; Bakan et al. 2003). These properties mean chronic elevations in MDA have been used as a marker of severity of oxidative stress and human disease (Romero et al. 1998). Although MDA is considered one of the most reliable markers of oxidative stress aside from isoprostanes, the measurement technique can be performed in a variety of ways that can greatly affect reliability. MDA is measured using the thiobarbituric acid (TBA) assay which relies on MDA reacting with TBA to form the TBA-MDA adduct which is then quantified. The principal problem with this assay is that many biological compounds can react with TBA, meaning a number of steps must be taken to reduce the possibility of cross-reaction and overestimation of MDA concentration. It is considered that including a lipid derivation step and using high-performance liquid chromatography (HPLC) greatly improve the accuracy of the assay.

Other more highly reactive markers of lipid peroxidation also exist which possess relatively short half-lives, but are involved in the aetiology of rapid cellular damage from oxidative stress (Fogarty et al. 2011). One such example is lipid hydroperoxides (LOOH) produced from the peroxidation of both saturated and unsaturated fats. LOOH are volatile and unstable, and can be produced from numerous mechanisms within biological systems (Powers, Smuder, et al. 2010). As such, measurement of LOOH is particularly valuable following a stimulus where a rise in oxidative stress is expected so that causality is relatively assured, and alongside a more stable measure of lipid peroxidation such as MDA. Like MDA, there are several methods for detecting LOOH but the most commonly used and reliable are ferrous ion assays that require several biochemical steps but are relatively inexpensive to perform.

Other markers of oxidative stress, such as aldehyde-protein conjugates and markers of protein oxidation are available that can be measured using more conventional methods such as ELISA or western-blot techniques but have a number

of disadvantages compared to those discussed above. For this reason these are less widely used in human physiology unless measured alongside a panel of other markers of oxidative signalling.

2.4.2 Smoking and oxidative stress

Typically, long-term habitual smokers exhibit chronically elevated systemic markers of oxidative stress in comparison to non-smokers (Morrow et al. 1995; Reilly et al. 1996; Helmersson et al. 2005; Isik, Ceylan & Isik 2007). It is theorised that this may be a mediating factor in the higher prevalence and earlier onset of chronic diseases in smokers (Ambrose & Barua 2004; Ahmadzadehfar et al. 2006; Faux et al. 2009). Cigarettes are known to contain substances that produce free radicals when combusted (Pryor 1997), directly affecting the oxidative environment within lung tissue. Substantial elevation in oxidation occurs in the lung tissue (Faux et al. 2009), causing an inflammatory-immune response. The first line of host defence is well equipped to manage an acute increase in oxidative stress. However, over repeated occurrences the high volume of ROS and particulate matter in smoke is suggested to vitiate lung tissue and function (Taylor 2010). Furthermore, smoking appears to attenuate the function of phagocytes within the lung (Hodge et al. 2007) and alter control of local inflammatory mediators (McCrea et al. 1994) whereby an increased production of oxygen radicals intended for immune defence are released into the surrounding environment (Cross et al. 1998; Gonçalves et al. 2011). These processes have a cascade effect on elevating oxidative stress and inflammation on a local and systemic level (Van der Vaart et al. 2004).

In fluid extracted from within the lungs, and from circulatory blood samples, oxidative stress is shown to transiently elevate following smoke exposure (Faux et al. 2009). However, smoking appears to influence different aspects of oxidative stress in the acute act of smoking than in response to habitual exposure (Seet et al. 2011). This was observed by measuring a panel of biomarkers, observing several to remain constant despite overnight abstinence from smoking, and others to respond transiently to smoking (Seet et al. 2011). As such, although the smoking of multiple cigarettes will maintain elevated diurnal oxidative stress, there is evidence to suggest that different aspects of oxidative stress contribute to the chronic state.

After prolonged regular smoking, habitual smokers develop chronic oxidative stress. This effect follows a dose-response, where concentrations of markers are proportional to average cigarette consumption (Reilly et al. 1996). This is evidenced by significantly elevated biomarkers of oxidative stress in individuals smoking >30 cigarettes.day⁻¹ (Reilly et al. 1996) in comparison to non- and moderate- smoking groups. In young otherwise healthy adults, oxidative stress markers reduce to levels similar to non-smokers after several weeks of cessation (Morrow et al. 1995; Pilz et al. 2000). Similarly, decreased *in vitro* oxidation was observed with one month of smoking cessation (Van den Bergmotel et al. 2000). However, it is understood that this response is inversely proportional to age and total duration of smoking, with the rationale that the ability to manage oxidative stress becomes impaired with continued smoke exposure. As such, elderly long-term smokers will likely exhibit chronic oxidative stress, and markers of ill health from smoking, for an extended period even following smoking cessation.

Smoking may also reduce the effectiveness of antioxidant defence. Concentrations of antioxidant micronutrients are reduced in comparison to non-smokers (Faruque et al. 1995; Alberg 2002), seemingly owing to higher oxidative stress levels and lower dietary antioxidant intake (Marangon et al. 1998; Bloomer 2007). Concordantly, the majority of studies show TAC is lower in smokers, although it has been suggested that persistent oxidative stress in smokers may in fact modify and increase TAC (Charalabopoulos et al. 2005). This was, however, accompanied by a reduced resistance of lymphocytes to hydrogen peroxide-induced damage, suggesting immunity was still impaired. The smoking-induced rise in oxidative stress produces an inflammatory-immune response, activating inflammatory mediators, signalling a local influx of phagocytes, leukocytes and monocytes. There is a resultant increase in systemic inflammation, which can also potentiate further oxidative processes (Cross et al. 1998).

2.4.3 Inflammation

Inflammation is a complex process within the human immune system characterised by systemic or local influx of a cell cascade in response to infection or tissue damage. It is generally considered that a short term inflammatory response is

necessary for the process of repair, where inflammatory mediators and signalling molecules travel to the site of insult. Molecules called cytokines are produced in the cascade to signal the release of immune cells such as neutrophils, monocytes, phagocytes and macrophages for neutralisation of foreign microbes. Appearance of cytokines in the circulation trigger the “acute phase response”, where acute phase proteins are produced, causing the influx of platelets, adhesion molecules and fibrinogen to sites of injury to begin the healing process (Ershler & Keller 2000; Arcavi & Benowitz 2004). Following a normal immune-inflammatory response, several anti-inflammatory processes are then initiated to counteract the elevated inflammatory state (Steensberg et al. 2003; Fischer 2006). Subsequent to completion of the healing process, markers of the process reduce to near undetectable levels.

Where the transient increase in inflammatory processes is considered beneficial to recovery, chronically elevated inflammation is associated with chronic disease states (Hirschfield & Pepys 2003). Plasma concentrations of inflammatory markers are elevated in individuals suffering from cardiovascular disease (Pearson et al. 2003), obesity (Ford 1999), cancer (Ono 2008) and osteoporosis (Mundy 2007). Basal low grade inflammation is pathologic in the development of atherosclerosis (Jialal, Devaraj & Venugopal 2004; Kuo et al. 2007) and coronary heart disease (Danesh et al. 2004). Resting markers of inflammation increase with age (Ershler & Keller 2000; Krabbe, Pedersen & Bruunsgaard 2004), supporting the premise that low-grade inflammation may also be involved in the ageing process.

2.4.3.1 Markers of inflammation

Owing to the clinical significance of inflammation, biomarkers of the process have been extensively researched. The pleiotropic cytokine interleukin(IL)-6 is a central marker in both pro- and anti-inflammatory processes (Scheller et al. 2011) as well as immune, neural and bone cell signalling (Kurihara et al. 1990; Tamura et al. 1993). IL-6 stimulates the production of several hepatic proteins as part of the acute phase response (Ershler & Keller 2000). Alongside this, IL-6 has immunological function, stimulating differentiation of B cells (Lotz et al. 1988), subsequently activating lymphocytes (Lotz et al. 1988; Luger et al. 1989).

Research has suggested IL-6 is predominantly a myokine, produced from skeletal muscle during contraction (Pedersen & Febbraio 2008). However, like its functions, the sources of IL-6 are numerous, with observations of hepatic production as well as from adipose tissue (Giannopoulou et al. 2005; Pedersen & Febbraio 2008). Consequently, elevations in acute phase proteins, neutrophils and macrophages follow circulatory increases in IL-6 (Ershler & Keller 2000; Febbraio & Pedersen 2002). Subsequent to this, IL-6 triggers anti-inflammatory markers IL-1ra and IL-10 (Steensberg et al. 2003). As such, IL-6 can be observed as a single central signalling molecule that can give a reliable representation of the current inflammatory state of an individual.

Interleukin-6 stimulates the production of C-reactive protein (CRP) from the liver (Ershler & Keller 2000). As an acute phase protein CRP is present in very low levels in the circulation and increases substantially as part of the inflammatory cascade. In instances of severe infection, CRP has been observed to increase in concentration by up to 10000 times (Hirschfield & Pepys 2003). In a clinical setting, CRP is used as a marker of atherothrombotic events, infection or illness and is typically proportional to severity. Clinical thresholds have been established (Clyne & Olshaker 1999), where chronic concentrations of CRP are risk factors of disease and can be predictive of future events (Ridker et al. 1998; Ridker et al. 2002). CRP has been linked to endothelial cell dysfunction (Fichtlscherer et al. 2000), formation of foam cells and activation of vascular smooth muscle, which over prolonged periods accelerate atherogenesis (Jialal et al. 2004; Kuo et al. 2007). Median population resting values range between $0.8\text{--}2\text{ mg}\cdot\text{L}^{-1}$, with $>3\text{ mg}\cdot\text{L}^{-1}$ being clinically indicative of increased cardiovascular disease risk, and $>10\text{ mg}\cdot\text{L}^{-1}$ during acute infection or immune stress (Clyne & Olshaker 1999; Woloshin & Schwartz 2005). In cases of severe burns or sepsis, CRP can rise as high as $200\text{ mg}\cdot\text{L}^{-1}$ (Clyne & Olshaker 1999; Luzzani et al. 2003).

Ordinarily, in acute inflammation elevations in CRP and IL-6 are concurrent (Sipe 1990). However, owing to a longer half-life in CRP, following an inflammatory response CRP remains in the circulation longer (Heinrich, Castell & Andus 1990). As such, the duration of time after an inflammatory stimulus is integral for drawing accurate conclusions from the measurement of these markers. Factors such as stress, sleep disruption, recent physical activity or illness, can affect

both pro- and anti- inflammatory processes (Irwin et al. 2006; Plaisance & Grandjean 2006; Frey, Fleshner & Wright 2007; O'Connor & Irwin 2010). During states of chronic inflammation, however, IL-6 and CRP can be elevated independent of one another (Dixon et al. 2009).

Other markers of inflammation exist that respond acutely to an inflammatory stimulus or as part of immune control. Often these markers have clear pro- or anti-inflammatory functions. Tumor necrosis factor (TNF) – α and IL-1b are typically the first molecules to be present in the circulation to initiate the systemic inflammatory response and the acute phase cascade (Bruunsgaard 2005). As discussed earlier, when the inflammatory response is transient these cytokines are then followed by anti-inflammatory markers such as IL-1ra and IL-10 in order to return inflammation to normal levels. These markers can be measured using commercially available assays. However, owing to their transient nature, when an inflammatory rise is not present the majority of these markers can be undetectable in human tissue and present considerable inter-individual variability.

Lastly, liver transaminases, such as alanine aminotransferase (ALT), have received research attention as novel indicators of cardiovascular disease risk (Yamada, Tomiyama, et al. 2006; Mochizuki et al. 2012). Specifically, when elevated, ALT can be indicative of liver disorders and has been positively associated with inflammatory biomarkers and cardiovascular disease risk in middle aged men (Yamada, Tomiyama, et al. 2006). Alongside CRP, ALT is significantly elevated in individuals with coronary artery disease (Masoudkabar et al. 2011).

2.4.4 Smoking and inflammation

Smoke exposure induces local and systemic elevations in primary inflammatory cells. Inflammatory cytokine release is evident in the airways and lungs in the hour following smoking (Flouris et al. 2009). However, smoke exposure suppresses the effectiveness of the immunological response. In bronchoalveolar lavage fluid of young smokers, despite a greater population of immune cells when compared to never-smokers, cytokine signalling and phagocytic

ability of macrophages is suppressed (McCrea et al. 1994; Hodge et al. 2007). Similarly, smoke exposure in mice results in elevations in cytokines accompanied with immunosuppression (Gualano et al. 2008). Effective macrophage function in lung tissue is pivotal for mediating inflammation and preventing excessive oxidative stress following smoking. However, in this state, the protective mechanisms of neutrophils and macrophages against particulate matter and tissue damage associated with smoking are impaired, resulting in further harm to tissue by release of oxygen species into the surrounding cellular environment (Cross et al. 1998; Gonçalves et al. 2011). This leads to a dose-response relationship between smoke exposure and cytokine concentrations observed in lung tissue (Kuschner et al. 1996).

The combined accumulation of oxidative stress and inflammatory mediators in the smoke exposed lungs elicit a systemic response. In smokers, it is unknown whether the inflammatory rise is solely as a result of oxidative stress. Additionally it has also not been elucidated what proportion or aspect of the oxidative state results from cigarette smoke and what results from the immune-inflammatory response. However, chronic low-grade inflammation, similar to that of chronic disease states, is typically observed in smokers (Andelid et al. 2007; Jang et al. 2007). Additionally, Helmersson et al (2005) demonstrated increased circulatory IL-6 and oxidative stress marker prostaglandin $F_{2\alpha}$ in tandem. Large scale studies have reported elevated levels of CRP in smokers in middle aged and elderly populations (Bazzano et al. 2003; Levitzky et al. 2008), as well as young otherwise healthy smokers (O'Loughlin et al. 2008) when compared to age-matched never-smokers. Similar results have been found in cross-sectional studies alongside elevated white blood cell count and fibrinogen (Tuut & Hense 2001; Wannamethee et al. 2005), further associating smoking with cardiovascular disease risk and reduced vascular health.

The direct link between smoking and chronically increased inflammation is starting to be revealed with further understanding of gene expression and modification in habitual smokers. The interaction between smoking and the IL-6 promoter gene has been observed and is related to the systemic elevation in CRP and IL-6 in smokers in a dose-dependent manner (Sunyer et al. 2009). Further to this, evidence exists for particular genotypes to be more susceptible to a greater IL-

6 response from smoking (Jang et al. 2007; Shin et al. 2007). As will be discussed later, the role of oxidative stress in mediating transcription factors of inflammatory cytokine release is evidenced (Close et al. 2005). Interaction of these processes provides the basis for how concurrent elevation of inflammation and ROS on a systemic level in smokers can culminate in local tissue dysfunction, as described in skeletal muscle and endothelial cells (Barreiro et al. 2010; Barbieri et al. 2011).

2.4.5 Smoking and hormones

Smoking and smoking-induced inflammation have a profound effect on circulatory hormone concentration (Kapoor & Jones 2005; Steptoe & Ussher 2006). Hormones are chemical messengers synthesised by endocrine glands and secreted into the circulation to be transported to a specific target tissue. Tissue responsiveness to hormones is determined by receptors on the destination cell surface or within the cytoplasm. Different endocrine glands each produce and secrete specific hormones in response to signalling from neural input, and the presence of other hormones or cells in circulation. The influence of chronic smoking on hormones is largely from the pharmacological effects of nicotine or subsequent to physical processes stimulated by smoking, such as increased heart rate. Consequently, habitual smokers have been shown to have altered hormone concentrations in comparison to typical values in non-smokers, specifically resting levels of testosterone (Zmuda et al. 1997), cortisol (Kirschbaum, Wüst & Strasburger 1992) and insulin-like growth factor(IGF)-1 (Renahan et al. 2004), amongst others (Perkins & Fonte 2002; Jorde et al. 2005).

Insulin-like growth factor-1 is a peptide hormone produced predominantly from the liver, stimulated primarily by growth hormone, but is also locally produced by numerous tissues within the body. Alongside roles promoting growth and development especially during maturation, IGF-1 has purported functions in maintenance of soft tissue and bone (Yakar et al. 2001; Juul 2003), glucose homeostasis and regulation of metabolism (Nindl & Pierce 2010). Long-term smoking is associated with a decline in IGF-1, ostensibly in proportion to cigarette exposure (Landin-Wilhelmsen et al. 1994). It appears smoking also may reduce production of circulating binding proteins (Kaklamani et al. 1999; Holmes, Pollak

& Hankinson 2002). It has been suggested that the likely mechanism for reduced IGF-1 concentration in smokers is reduced growth hormone, with Kapoor & Jones (2005) postulating this would be mediated by central effects on the hypothalamus.

The effect of nicotine acting on the hypothalamus is also the proposed pathway for smoking-induced increase in cortisol. Nicotinic binding sites on the hypothalamus (Kellar, Dávila-García & Xiao 1999), by stimulating corticotrophin releasing hormone and adrenocorticotrophic hormone increase cortisol secretion in smokers (Steptoe & Ussher 2006). Cortisol concentration in saliva (Kirschbaum et al. 1992; Badrick, Kirschbaum & Kumari 2007) and serum (Field et al. 1994) is greater in chronic smokers, and decreases in response to long-term smoking cessation (Frederick et al. 1998). Some studies have shown no differences in cortisol between smokers and non-smokers (Kirschbaum, Scherer & Strasburger 1994; Tsuda et al. 1996). However, as cortisol follows a circadian rhythm, the discrepancies in these studies may stem from varied timing of blood sampling (Steptoe & Ussher 2006). Alongside these mechanisms both IGF-1 and cortisol are down- and up-regulated, respectively, by the appearance of IL-6 in the circulation (Steensberg et al. 2003; Joseph, Kenny, et al. 2005).

In comparison to IGF-1 and cortisol, the effect of smoking on testosterone is more equivocal. Testosterone is a steroid hormone produced in the testes in men and ovaries in women, but can also be secreted, to a lesser extent, from the adrenal gland. Researchers examining the effect of habitual smoking on testosterone concentration have observed mixed results, with some authors reporting greater (Gray et al. 1991; Field et al. 1994; Svartberg et al. 2003; Svartberg & Jorde 2007), others reporting lesser (Zmuda et al. 1997), or others reporting no measureable difference (Harman et al. 2001; Richthoff et al. 2008) when compared to non-smokers. There are a number of interlinked explanations for these inconsistencies that appear from the literature (Gray et al. 1991; Kapoor & Jones 2005). Studies have measured a variety of fractions of testosterone in serum and saliva, namely free, bioavailable or total testosterone, which may account for some of the variation in results. Examination of the methods for measuring testosterone has indicated there are a number of differences in assays and blood sample timing that are pivotal for correctly determining testosterone concentrations (Gray et al. 1991). The majority of circulatory testosterone (65-80%) is bound to sex hormone binding

globulin (SHBG), and is therefore biologically inactive. Another 20-40% is loosely bound to albumin, and is available for receptor binding, while a final ~2% is the free fraction. It is understood that the combination of albumin bound and free fractions make up the bioavailable fraction, but this definition is made unclear by assay variation. Several assay types will cause the unbinding of testosterone from albumin and SHBG ostensibly measuring the total concentration of testosterone, where some claim to directly measure bioavailable testosterone, and others employ a calculation to estimate bioavailability from the albumin bound fraction. Similar to cortisol, testosterone follows a diurnal rhythm meaning that not only bioavailability could differ, but total concentration may differ depending on timing of blood sampling.

2.5 Physiological mediation of physical adaptation to training

Oxidative stress, inflammation and aspects of endocrine status were introduced in section 2.4 alongside how these can be influenced by habitual smoking. Research examining the biomarkers presented above has observed their acute responses to exercise and the potential roles of redox balance, inflammation and hormones in adaptation to exercise training. In this section, the extant evidence for how these factors may respond to exercise and potentially mediate physical adaptation to long-term exercise training will be presented. The enhancement of muscle strength, size, fatigability and contractility via adaptation to exercise requires a balance of physiological processes that mediate protein degradation and protein synthesis, the availability of nutrients, and the proliferation of satellite cells for alterations in muscle cell properties, with subsequent remodelling of muscle. The optimal cellular environment for these adaptive responses and for effective recovery from exercise is constantly re-defined. Given evidence discussed in section 2.4, it is proposed that the process of adaptation may be disrupted by chronic levels of oxidative stress, inflammation and alterations in hormone balance in a habitually smoking individual.

2.5.1 Oxidative stress and exercise training

Exercise causes an increase in the presence of ROS in the circulation, with specific complexes of the electron transport chain within muscle mitochondria shown to be the predominant source of superoxide during exercise (Barja 1999; Muller, Liu & Van Remmen 2004). There is evidence of oxidant release and oxidative enzymes in the sarcoplasmic reticulum (Xia et al. 2003), transverse tubules (Hidalgo et al. 2006) and subcellular sources within muscle cells (Powers & Jackson 2008), demonstrating how muscular contractions can increase local generation of ROS (McArdle, Van der Meulen, et al. 2004). Though originally thought to be potentially harmful to muscle cells, the understanding of the role of oxidants in muscle has greatly advanced. It is now known that transient elevations in ROS during exercise are favourable for muscle function. By incubating isolated animal muscle in antioxidant enzymes, oxidative intermediates have been shown to be essential for optimal muscle contraction in un-fatigued muscle (Reid, Khawli & Moody 1993). It was speculated that oxygen radicals are obligatory for excitation-contraction coupling (Reid et al. 1993). This was later demonstrated by antioxidants impairing contractile ability by lowering ROS in the muscle cell cytosol (Reid & Moylan 2011).

Alongside the immediate functional benefits on muscle contraction, oxygen species may also have an effect on muscular adaptation to exercise. The work of Close and colleagues (2005) on the potential role of ROS in muscle damage proposed that cellular oxidation does not exacerbate the magnitude of damage and instead appears to signal an adaptive response. Evidence shows that ROS signal transcription factors that modulate the expression of specific genes in response to contraction (Ammendola et al. 1995; Lander et al. 1996). Associated genes, such as NFkB, are identified to have pivotal roles in the modulation of inflammatory mediators and the generation of antioxidant enzymes (Close et al. 2005). Furthermore, the transcription factors and associated inflammatory response positively influence cell regeneration (Jackson et al. 2002; McArdle, Vasilaki & Jackson 2002), potentially repairing oxidative damage and improving muscle health. As such, transient increases in oxygen radicals appear to trigger an adaptive response to modify the system to more effectively manage future oxidative states.

In the long term, high-intensity endurance training appears to result in more prolific generation of antioxidant enzymes and intermediates (Sen et al. 1992), specifically glutathione in muscle cells (Marin et al. 1993; Leeuwenburgh et al. 1997). The adaptive response to oxidative events in muscle also contains protective mechanisms against future cellular damage (Ji 2007), in part via expression of heat shock proteins (McArdle, Dillmann, et al. 2004) and cytoprotective proteins (McArdle et al. 2001). With examination of repeated contractions, the oxidation required to trigger these responses is reversible within 60 minutes (McArdle et al. 2001), indicating the transient nature of beneficial oxidative stress in muscle.

Reactive oxidants, however, have different functions depending on the state of the muscle tissue. The situation observed in un-fatigued muscle as discussed above differs from the cellular environment during persistent exhaustive contractions. Here, the accumulation of ROS is substantial enough to result in a prolonged state of oxidative stress. The progressive insensitivity to calcium which occurs in fatiguing muscle is accelerated by the oxidative state, altering contractile ability (Andrade et al. 1998; Moopanar & Allen 2005) and oxidising contractile myosin (Yamada, Mishima, et al. 2006). Andrade et al. (1998) observed improvements in force production in isolated myofibrils with the presence of hydrogen peroxide for brief periods, but prolonged exposure significantly decreased force. Similarly, repeated bouts of modest peroxide concentrations did not hinder pathways for contraction, allowing force to remain relatively constant over time (Andrade, Reid & Westerblad 2001). In the same experiment, solely the highest concentrations of peroxides examined decreased calcium uptake and increased resting in mouse muscle fibres (Andrade 2001). These findings allude to a concentration-dependent limit of oxidative stress in muscle, as opposed to a dose-response relationship, whereby there is an upper-limit of the presence of ROS beneficial for contractile function.

Given the existing literature, it would seem that it is the redox balance within muscle cells that is not only important for optimal myofibrillar function, but for the successful signalling of protective adaptation to exercise (Jackson 2009; Jackson & McArdle 2011). Within human physiology, the direct correlation between circulatory biomarkers and their accumulation within local tissues is equivocal. However, it is reasonable to suggest that chronically elevated circulatory

oxidative stress in smokers may disrupt levels of cellular oxidants. This may highlight potential causation behind reduced fatigue resistance in muscle with smoke exposure (Morse et al. 2007; Wüst et al. 2008). With a graded exercise test, by comparing pre- and post-exercise levels of MDA in smokers and non-smokers, Bloomer et al. (2007) identified that smokers experienced a significantly greater oxidative stress response. The chronic oxidative state of smokers may facilitate the progression, or shorten the time needed, to reach the upper limit of oxidation in the cellular environment. Equally, as smokers typically display lower antioxidant levels than non-smokers, the ability to sustain high intensity exercise may be reduced. As such, it is proposed that elevated endogenous ROS in smokers may impair the adaptive responses of muscle to exercise training in comparison to non-smokers, either by altering the short term function of muscular contraction or disrupting cellular pathways of signalling for adaptation.

2.5.2 Inflammation and exercise training

Long-term physical training has an anti-inflammatory effect on resting inflammation (Petersen & Pedersen 2005). Endurance exercise interventions lasting from 3-9 months have reduced markers of resting inflammation in middle-aged sedentary populations (Mattusch et al. 2000; Giannopoulou et al. 2005; Thompson et al. 2010), although others have seen no effects (Marcell et al. 2005; Nakajima et al. 2008). Furthermore, longitudinal data has shown that inflammation is inversely proportional to regular physical activity level (Mattusch et al. 2000; Fallon, Fallon & Boston 2001; Dixon et al. 2009) and endurance capacity (Kuo et al. 2007). This supports the prescription of exercise as a method of controlling resting inflammation (Mathur & Pedersen 2008).

Exercise stimulates a transient elevation in inflammation. Typically, IL-6 and other inflammatory cytokines are observed in greater numbers in circulation immediately following exercise (Febbraio & Pedersen 2002). Muscle-derived IL-6 is produced during muscular contraction, with substantial increases shown following maximal rowing (Nielsen et al. 1996), and marathon running (Ostrowski, Schjerling & Pedersen 2000; Ostrowski et al. 2001). The extent to which IL-6 increases appears to be influenced by the duration and intensity of exercise

(Pedersen et al. 1998). Greater cytokine responses were observed to 60 min of running at 75% $\dot{V}O_2$ max compared to 65% and 55% $\dot{V}O_2$ max (Scott et al. 2011), eliciting greater time to return to pre-exercise levels. Equally, IL-6 response appears to be proportional to duration when performing steady state exercise (Febbraio & Pedersen 2002; Fischer 2006). This elevation in IL-6 gives rise to CRP which can continue to develop over approximately 24 hours following exercise (Mendham et al. 2011). Particularly unaccustomed exercise and severe muscle damage can result in consecutive days of elevated CRP, although this may be partly attributed to a delayed response of IL-6 to eccentric exercise (MacIntyre et al. 2001).

Similar to the immune response to infection and injury, on a transient basis an elevation in inflammation is perceived to be beneficial to adaptation and recovery from exercise in comparison to a chronic elevation. The difference being that the potent pro-inflammatory cytokines that elevate in response to infection are not stimulated by exercise, and instead IL-6 is the first inflammatory mediator observed in circulation (Mathur & Pedersen 2008). However, chronic low-grade inflammation, as observed in chronic disease states, up-regulates pro-inflammatory cytokines and is implicated in the local accumulation of cytokines in muscle and subsequent mechanisms in disease-induced muscle atrophy (Ershler & Keller 2000; Schaap et al. 2006). In longitudinal studies both resting CRP and IL-6 levels are shown to be inversely proportional to muscle strength and cardiovascular fitness in elderly populations (Taaffe et al. 2000; Church et al. 2002; Visser et al. 2002; Hamer & Molloy 2009).

Acute increases in IL-6 in muscle augments protein synthesis, while longer duration infusion has an opposing effect (Goodman 1994). Local infusion of IL-6 in rat muscle to levels comparable with chronic resting inflammation in elderly humans reduces growth factors and signalling for adaptation (Haddad et al. 2005). Equally, Bodell et al. (2009) observed that a rat infusion model of IL-6 resulted in 13% lower content of myofibrillar protein and lower body mass during growth. Although these studies examined rats during natural growth, muscle biopsies in healthy adult men following infusion of IL-6 for three hours exhibited 50% reduced protein synthesis compared to controls (Van Hall et al. 2008). This may explain resting levels of IL-6 and CRP being negatively associated with protein synthesis

rates (Toth et al. 2005). It has also been shown that IL-6 has a key role in the regulation of growth factors in circulation as well as their actions on the maintenance of muscle (Steinacker et al. 2004; Fischer 2006; Adams 2010), which will be discussed in section 2.5.3.

A positive adaptation to long-term exercise is the concurrent reduction in resting inflammation. The persistent elevation of inflammation within the circulation elicits numerous subsequent effects that may induce damage to muscular cells and delay recovery (Tidball 2005). Elongation of the transient exercise-induced inflammatory response increases the appearance of pro-inflammatory cytokines, several inflammatory mediators and neutrophils (Pedersen et al. 1998; Bruunsgaard 2005). The role of neutrophils following muscle injury or damage is to clear damaged tissue by release of cytotoxic molecules, for development of new tissue, but this can damage healthy muscle cells (Tidball 2005). Additionally, although the molecular mechanisms underlying proliferation and differentiation of muscle satellite cells are ill-defined, it has been suggested that these may involve the balance of inflammatory cells in the muscle cellular environment (Sun et al. 2007).

2.5.3 Hormones and adaptation to exercise training

It has been discussed how both redox balance and inflammatory mediators can be influential in the process of adaptation to training. It appears that there may be a role of hormones in mediating adaptation and recovery to exercise training, but is continually under discussion. While it is clear both in neonates and during growth and maturation, that growth factors and anabolic hormones such as IGF-1 and testosterone have necessary functional roles in the development of tissue, the significance of these roles in training response later in life and on a diurnal basis are argued.

In acute response to both low- and high-intensity exercise, IGF-1 has predominantly been shown to remain unchanged (Meckel et al. 2009; Stokes et al. 2010; Wahl et al. 2010), and exercise training research focuses on more long-term temporal alterations in IGF-1 and its binding proteins. In cross sectional studies,

IGF-1 has been associated with better health outcomes, greater physical fitness and strength (Cappola et al. 2001; Nindl & Pierce 2010; Nindl et al. 2011), and maintenance of muscle (Baumgartner et al. 1999) and bone mass (Joseph, Kenny, et al. 2005). IGF-1 is also involved in the regulation of metabolism, specifically via maintenance of glucose homeostasis and insulin sensitivity (Yakar et al. 2001). It is this reason that energy balance and dietary intake can exert considerable effect on IGF-1 concentration (Nindl & Pierce 2010). As such, during consecutive days of training involving energy deficit IGF-1 becomes significantly reduced (Nindl, Barnes, et al. 2007) and specific binding proteins can be useful markers of overtraining (Elloumi et al. 2005). As such, a reduction in IGF-1 over time has been considered an indicator of metabolic or physiological stress (Nindl, Alemany, et al. 2007).

Animal models have shown the importance of IGF-1 in development of tissue during growth and maturation, and in maintenance of muscle in more advanced ages. Locally produced IGF-1 in muscle has been shown to prevent muscle mass decline in degenerative mice (Barton et al. 2002), induce hypertrophy (Musrò et al. 2001) and maintain regeneration of muscle fibres in healthy mice (Musrò et al. 1999). Similarly, Adams & McCue (1998) infused IGF-1 into rat muscle and reported a 9% greater total mass compared to the contralateral muscle. However, where another mouse model showed greater muscle hypertrophy in animals with greater IGF-1, this result was only reported in muscles during normal pre-natal growth (Shavlakadze et al. 2010). It is this evidence that causes the role of IGF-1 as a major regulator of muscle mass in fully matured humans to be debated (Stewart & Pell 2010).

In humans, the balance of IGF-1 and IGF-binding proteins are modulated following resistance exercise to promote maintenance of muscle by increasing bioavailability (Izquierdo et al. 2006). There is also a chronic training response of IGF-1, where the modulation of IGF-1 binding proteins in response to training differs between well-trained and untrained individuals (Rosendal et al. 2002).

The interaction of IL-6 with IGF-1 is also important for adaptation and development of muscle tissue. Several pathways have been shown whereby cytokine signalling and IGF-1 mediate one another (Adams 2010). Lieskovska et

al. (2002) observed that over expression of IL-6 caused down-regulation of IGF-1 and an impairment of growth. Furthermore, the process of muscle regeneration is accelerated by the signalling of cytokines by IGF-1 (Pelosi et al. 2007). While it has been shown that hypertrophy stimulated from mechanical loading can be achieved without the presence of IGF-1 (Spangenburg et al. 2008), it would appear that the role of IGF-1 is pivotal in the effectiveness and rapidity of muscular regeneration and maintenance of muscle mass via mediation of inflammatory processes (Clemmons 2009).

Testosterone and cortisol are widely regarded as modulators of skeletal muscle remodelling (Crewther et al. 2011). With a role in promoting protein synthesis and decreasing protein degradation, testosterone is termed an “anabolic” hormone. The chief role of cortisol on metabolism is the stimulation of gluconeogenesis and glycogenolysis, but also acts to increase degradation of protein and reduce protein synthesis, thereby primarily catabolic in nature. As such, the balance between the two is considered an indicator of tissue state of health. In acute response to exercise, concentrations of both hormones typically elevate (Hayes, Bickerstaff & Baker 2010; Vingren et al. 2010; Cadore et al. 2012).

In animal models, the removal of testosterone secretion results in a reduction in muscle strength (Brown, Fisher & Hasser 2001), and muscle mass is regained with testosterone supplementation (Krotkiewski, Kral & Karlsson 1980). The exogenous use of testosterone induces hypertrophy in animals (Ustünel, Akkoyunlu & Demir 2003) and in young (Bhasin et al. 2001) and elderly men (Sinha-Hikim et al. 2006). These relationships are characterised by greater muscle fibre cross sectional area, reduced muscular fatigue, elevated proliferation of satellite cells and greater improvement in fat-free mass than controls (Bhasin et al. 1997; Bhasin et al. 2001; Sinha-Hikim et al. 2002; Axell et al. 2006; Sinha-Hikim et al. 2006). In longitudinal and cross-sectional studies, testosterone is positively associated with greater muscle mass (Baumgartner et al. 1999). As suggested, cortisol has an opposing effect to testosterone on muscle metabolism, inducing a reduction in protein synthesis rate 18 hours following infusion (McNurlan et al. 1996), meaning a decrease in cortisol should reduce the magnitude of degradation in muscle cells during training.

However, these studies have shown positive training effects with exogenous testosterone supplementation or loss of muscle mass with testosterone inhibition. Research observed that training eliciting high and low acute elevations in endogenous testosterone produced no differing effect on protein synthesis (West et al. 2009). As such, the emergent theory is that within normal physiological levels, hormones that mediate muscle growth work on a permissive basis, and that transient elevations are not effective in a dose-response manner (West et al. 2010). Therefore, it may be that resting basal levels of testosterone and cortisol are more relevant for how effectively adaptation will occur during a period of training. Chronic increases and decreases in resting testosterone and cortisol have been observed in response to periods of training in trained (Kraemer et al. 1998; Kraemer et al. 1999; Ahtiainen et al. 2004; Kraemer et al. 2006), but not untrained individuals (Ahtiainen et al. 2004; Crewther et al. 2011). Unfortunately, it has not been made clear how well changes in performance are concurrent with alterations in resting levels of these hormones, and therefore the possible implications on muscular adaptations to exercise.

In light of the extant literature presented above, it appears that physiological effects of smoking have been recognised in middle-aged and elderly populations, usually with the aim of discussing health outcomes, but whether these effects exist in young, active populations is not clear. It would be of interest to determine whether smoking impairs development of physical fitness. Furthermore, it would be worthwhile to examine oxidative stress, inflammation and hormonal markers alongside performance adaptation during training in case potential underlying mechanisms for any observed effects of smoking on adaptation are highlighted. Given the proposed roles oxidative stress, inflammation and endocrine status upon adaptation, examining any differences at rest during training or in acute response to exercise between smokers and non-smokers may be prudent. It is clear that smoking habits in military training populations are highly variable but it is possible that if prevalence is higher than in the public general public any adverse effects of smoking may affect a substantial number of trainees. As such, determining the

smoking habits within a military trainee cohort alongside physical fitness parameters and risk of injury in smokers compared to non-smokers could provide a comprehensive profile of how smoking can affect military training populations, with potential relevance to other young, otherwise healthy smoking populations.

CHAPTER 3

General Methods

General Methods

3.1 Study location

Data collection for the work in this thesis was completed between the months of September 2008 and July 2011 at Infantry Training Centre, Catterick (ITC(C)), UK.

3.2 Participants

All participants involved in the research were male trainees either undertaking the 26-week Combat Infantryman's Course (CIC) (Studies 1-4 and 6) or the 26-week Parachute Regiment Course (Study 5) at ITC(C). Inclusion criteria were the same as successful admission into the military course, meaning participants were aged between 17 and 33 years and successfully passed the week 1 Army medical examination.

3.3 Military training and experimental control

During this programme of research participants followed the normal training syllabus with only minor modifications to allow for data collection where necessary. All of these modifications were discussed with platoon staff to ensure normal practice was not affected.

Despite this programme of work being field-based, the military training environment does introduce standards of experimental control that are not feasible in free-living work. Military trainees follow a strict timetable that ensures that in each training week each platoon will typically experience identical waking and meal times. Similarly, standardised menus and canteens on the military camp limit potentially substantial variability in diet composition. Equally, during military training exercise is also largely standardised and completed in classes or groups, meaning it is unlikely for trainees to participate in more or less exercise training than their counterparts in a given training week.

With particular relevance to smoking behaviour and environmental tobacco exposure (passive smoking), it should be noted that this research took place following the introduction of the UK smoking ban, meaning smoking could only take place outside in designated smoking areas at a specific distance from places of work. At ITC(C), military trainees were allowed out to specific shelters if a cigarette break was deemed appropriate. This means those who did not smoke were unlikely to be subject to many instances of passive smoking.

3.4 Notes on ethical considerations

All studies in this programme of work were approved by the Ministry of Defence Research Ethics Committee. All trainees gave informed consent to take part in the research (Appendix A). Throughout the research every effort was made to dispel social bias or military and environmental pressure on individuals to take part. All smaller scale population studies (Studies 2, 3 and 5) were preceded by a verbal brief of the research project by a member of the project team without the presence of military staff, and at each time point participants were reminded that the participation in the research was voluntary and would not in any way affect their military careers. In the larger scale epidemiological studies (Studies 1, 4 and 6) administration of a questionnaire to an annual trainee intake by project investigators was not possible. As such, project researchers briefed select members of military staff on how to explain the nature of the research. These staff members were then observed administering this brief and the associated questionnaire to trainees on several occasions throughout the duration of research.

3.5 Smoking behaviour

3.5.1 Smoking/tobacco exposure and cigarette consumption

Unless otherwise stated, during this thesis smoking or tobacco “exposure” refers to the estimated lifetime exposure to tobacco in pack-years, calculated by cigarettes smoked daily divided by 20 (1 pack), multiplied by years smoked. The

term “cigarette consumption” refers to rate of cigarette consumption in average cigarettes per day.

3.5.2 Lifestyle questionnaire

Volunteers for all studies were asked to complete the Military Pre-training Questionnaire (MPQ) to determine individual smoking characteristics (Appendix B). The questionnaire was completed in a suitably quiet environment during the first physical fitness session during week 1 of training. In Study 5 the questionnaire was also administered in the final week of training of the Parachute Regiment. The MPQ was designed to assess risk factors for training-related injury in the military. Previous testing using weighted kappa analysis for reliability and validity found all smoking questions to have between “substantial” (0.6-0.8) and “near perfect” (0.8-1.0) strength of agreement in test-retest reliability scores (Robinson et al. 2010). For the purpose of this research the questionnaire recorded details on smoking status, smoking history and smoking behaviour prior to joining the army. For Study 4 physical activity constructs included in the MPQ were used to give an estimate of physical activity prior to entry to training. For use in other research projects not attached to this thesis the questionnaire also collected data on injury history, diet and alcohol intake. Questions consisted mainly of multiple choice tick box answers with some questions asking for a numerical value. The questionnaire has a Flesch Reading Ease Score of 70.1% and a Flesch-Kincaid Grade Score of 7.0, which is equivalent to a UK reading age of 12. In order to ensure accuracy and validity, all questionnaires were equipped with a security strip to conceal identification and maintain anonymity until collected by study investigators. It was also made clear to participants in the verbal brief and on the front cover of the questionnaire that results would not be seen by military staff and would not in any way influence their military careers.

3.5.3 Smoking status

Questionnaire items were designed to allow clear distinctions between habitual current smokers, occasional smokers, former smokers and non-smokers

from participant responses. Regular smokers were defined by those who answered item Q5-4 (“How often do you smoke at the moment?”) by marking the box “Regularly (at least one cigarette/roll-up a day)”. Occasional smokers were defined as those who answered item Q5-4 by marking the box “Occasionally (less than one cigarette/roll-up a day)”. Former smokers were defined by those who answered “No” to item Q5-3 (“Do you smoke cigarettes at all now?”) and subsequently answered “Yes” to Q5-11 (“Did you use to smoke regularly?”). Non-smokers were defined as those who answered “No” to item Q5-1 (“Have you ever smoked a cigarette (including roll-ups)?”) or “No” to both items Q5-3 (“Do you smoke cigarettes at all now?”) and Q5-11 (“Did you used to smoke cigarettes regularly?”).

3.5.4 Smoking groups

In studies 2, 3 and 6 current smokers were split into subgroups based on reported number of cigarettes smoked per day (cigarette consumption). The light smoking (LS) group comprised trainees reporting 1-9 cigarettes per day, moderate smoking (MS) 10-19 cigarettes per day and heavy smoking (HS) by ≥ 20 per day. These groups agree with the predominance of research that categorise light smokers as those smoking below the mode cigarette consumption of 10, and heavy smokers as those smoking 20 or more cigarettes per day (Bernaards et al. 2003; Munnoch & Bridger 2007)

3.5.5 Follow-up

A shortened version of the MPQ, solely containing items related to smoking, was administered in studies 1, 3 and 4 to capture any changes in smoking status between each time point. Further details are given in the methods sections of each study.

3.5.6 Estimated physical activity

The MPQ collected information on self-reported physical activity and exercise behaviour using constructs based on the Leisure Time Exercise Questionnaire (LTEQ). Physical activity was separated into three categories, light, moderate and vigorous for which respondents give an average frequency per week for each. A previously described weighting system was used to compile a total physical activity score from each respondent whereby the frequency of light, moderate and vigorous exercise behaviours were weighted by 1, 3 and 9, respectively, and summated (Godin & Shephard 1985).

3.6 Anthropometric data

3.6.1 Body mass and height

In studies 2, 3 and 5 anthropometric data were collected. Body mass and height were measured using a set of calibrated weighing scales accurate to ± 0.05 kg (Seca, Hamburg, Germany) and a stadiometer (Leicester, UK), respectively.

3.6.2 Estimated body fat percentage

In studies 2, 3 and 5 body fat percentage was estimated using measurements of skin fold thickness (Durnin & Womersley 1974) on four sites of the upper body (Biceps brachii, triceps brachii, sub-scapular and supra-iliac) using callipers (Holtain LTD., Crymych, UK). Waist circumference was also measured at the thinnest point of participant torso by tape measure.

3.6.3 Lower leg characteristics

In Study 3 muscle and fat characteristics of the lower leg were measured using peripheral quantitative computed tomography (pQCT). The dominant leg of each individual was used for the scan. Muscle area, fat area, muscle density and

total density (muscle and fat) were measured by pQCT. Fat to muscle area ratio was calculated from these data.

3.6.4 Lung function

Lung function was measured in studies 2 and 3 by spirometer (Micro Medical) for forced expiratory volume over one second (FEV₁), forced vital capacity (FVC), forced expiratory ratio ([FER= FEV₁/FVC)x100]) and peak expiratory flow (PEF).

3.7 Physical performance testing

In Study 3 all the following performance tests were completed. Platoons were given a full demonstration of each physical performance test in all visits. Verbal encouragement was given throughout and all data were recorded by the project researchers. Maximum values were recorded for all variables.

3.7.1 Maximal strength tests

Peak dynamic strength measures consisted of chest press, seated row and leg press exercises using a strength dynamometer (Concept2, Nottingham, UK). Participants completed five repetitions of chest and row exercises and ten repetitions of the leg press exercise, each with five seconds recovery between repetitions. Peak isometric hand-grip and static lift strength were measured using portable dynamometers (Takei, Japan). For static lift strength two attempts were completed and for hand-grip strength two attempts were completed for each side of the body.

3.7.2 Explosive power

Explosive power was measured by maximum counter-movement jump height using a jump mat (FSL, UK). Participants were given three attempts to jump as high as possible.

3.7.3 Army physical fitness test

The Army physical fitness test (PFT) is implemented at weeks 1, 14 and 24 of initial training and consists of a timed best effort 2.4 km run and the number of press ups and sit ups completed when allowing 2 minutes for each exercise. Trainees are allowed to rest at any point and can keep any desired pace until the distance or time-limit has expired. British infantry standards criteria for passing a PFT during training are a run time of under 10 mins 30 secs (630 secs) and greater than 44 press ups and 50 sit ups in two minutes. In Study 3 PFT results were collected for all trainees at weeks 1 and 14, and in Study 4 at weeks 1, 14 and 24.

3.8 Overtraining questionnaire

In Study 3, to assess overreaching or overtraining in trainees at weeks 1, 5 and 10, participants were asked to also fill in a training questionnaire (Brun, 2003). The questionnaire contained 54 items assessing mood and symptoms of overtraining. The method of assessment of potential overtraining employed by Brun (2003) was also followed, such that if one point were given for every “yes” a score of ≥ 25 would signify possible overtraining.

3.9 Blood sampling

In studies 2 and 3 blood samples were taken upon waking (0500-600) in trainee accommodation lines. Participants abstained from smoking or consuming food or drink in the morning until after blood samples had been collected. All blood samples were taken by venepuncture using a tourniquet from an antecubital vein using a needle and Vacutainer system (BD Diagnostics, Becton, Dickinson &

Co.). Each blood sample was collected using two plain untreated tubes (6 mL) and one tube (2 mL) containing EDTA. Samples in plain tubes were left to clot for 60 minutes before being spun to separate the serum. All samples were aliquoted and stored at -80°C for blood chemistry analysis. The 2 mL EDTA tubes were kept as whole blood for a full blood count analysis.

In Study 5 the same procedure was followed but samples were collected in 1 plain untreated tube (6mL), two tubes containing EDTA (6mL & 4mL) and one tube containing heparin (4mL). Additionally, in Study 5 post-exercise blood samples were collected at the site of exercise as soon as possible (within 15 mins) after exercise completion.

3.10 Biochemical analysis

3.10.1 Oxidative stress

Oxidative stress was determined in serum in studies 2 and 3. Malondialdehyde (MDA) was determined in serum following the HPLC method described by Funes et al. (2009). This method was based on the derivatization of MDA using 2-thiobarbituric acid (TBA), leading to the formation of the fluorescent MDA-TBA complex. 1,1,3,3-tetraethoxypropane (TEP) in 1% H₂SO₄ was used as stock standard solution and its concentration was determined using its absorbance at 244 nm and the molar extinction coefficient. Standards were prepared through serial dilution of the stock standard. 50 µL of serum or TEP standard were mixed with 50 µL of 0.05% butylated hydroxytoluene (BHT) in ethanol and 50 µL of trichloroacetic acid 20% in HCl 0.6 M. The samples were incubated on ice for 15 min and then centrifuged at 12,000g for 5 min at 4°C. Then for each sample 100 µL of the supernatant was added to 100 µL of TBA 0.6% in water in 2 mL capacity plastic centrifuge tubes, and the mixture was incubated at 97°C for 1 h in a dry incubator. Following heat derivatization, samples were placed in an ice-water bath for 5 minutes to cool. Subsequently, 300 µL of n-butanol was added to each sample to extract the MDA-TBA complex. Tubes were vortex mixed vigorously for at least 30 seconds then centrifuged at 10,000g for 3 min. Aliquots of 200 µL were removed from the n-butanol layer for HPLC analysis. The TBA-MDA chromogen was

determined using HPLC and a fluorescence detection system (Waters, Milford, USA). The analysis was conducted by injecting 10 μL of sample into a reverse phase column (Nova Pak, C18, 3.9x300 mm column) using isocratic mode with methanol: potassium phosphate buffer 50 mM, pH 6.8 (40:60, v/v), and a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$. The TBA-MDA product was monitored by fluorescence detection with excitation at 515 nm and emission at 553 nm.

The concentration of lipid hydroperoxides (LOOH) was determined in serum following the method described by Gay & Gebicki (2002) with slight modifications. This method was based on measurement of the ferric-xylenol orange complex in a perchloric acid medium (PCA-FOX assay). In a 1.5 ml micro-centrifuge reaction tube, 800 μL of water, 100 μL of serum and 100 μL of a solution containing 2.5 mM ferrous ammonium sulphate and 2.5 mM xylene orange in 1.10 M perchloric acid were vortex-mixed for 30 seconds. After 30 minutes at room temperature in the dark, samples were centrifuged at 12,000g for 5 min at 20°C. Then, for each sample 200 μL of the supernatant was removed and placed in a 96 well plate for absorbance reading at 560 nm in a microplate spectrophotometer (BIO-TEK, Power Wave XS). For each sample, a control containing 900 μL of water and 100 μL of serum was used. Freshly prepared H_2O_2 solution was used as the stock standard. The concentration of this stock solution was determined using the molar extinction coefficient at 240 nm.

It should be noted that all oxidative stress analysis was undertaken by a collaborator in an established research laboratory. All standards and samples were analysed in duplicate apart from LOOH which was analysed in triplicate.

It was the intention to also measure F_2 -Isoprostanes as a marker of oxidative stress in serum. Unfortunately, resources were not available for GC-MS analysis as intended. Subsequently, bench-top assays from two companies were tested with study samples but gave highly inconsistent results or values outside of physiological range. These inconsistencies may have been caused by technical issues with the assays or from incorrect pre-treatment of samples. With limited resources, it was decided that sufficient quantification of oxidative stress would be possible using analysis of MDA and LOOH to establish differences between smokers and non-smokers.

3.10.2 Inflammation

Inflammation was measured in serum in studies 2, 3 and 5. For chapter 5 in particular it is recognised that having analysis of pro- and anti-inflammatory cytokines would have been preferable for understanding the time-course of acute inflammation but resources and blood sample size were not sufficient to allow for further analytes. It was decided that measuring the cytokine IL-6 alongside the acute phase protein CRP would be sufficient to show the inflammatory status in the population.

Serum concentrations of IL-6 were also measured by commercially available enzyme immunoassays (R&D Systems Inc., Abingdon, UK). The manufacturer's reported sensitivity and intra-assay coefficient of variance were 0.04 pg.mL⁻¹ and 7.4%. Combined intra- and inter-assay coefficient of variance calculated from study assay data for IL-6 was 2.00%.

Serum concentration of CRP was measured by commercially available enzyme immunoassays (Diagnostic Systems Laboratories Inc., Webster, Texas, USA). The sensitivity and intra-assay coefficient of variance for this assay were 1.6 ng.mL⁻¹ and 2.8%. Combined intra- and inter-assay coefficient of variance calculated from study assay data for CRP was 2.22%.

Serum Alanine transaminase (ALT) was examined in order to assess liver health which may alter the production of inflammatory markers. ALT was measured using a commercial assay (Randox Laboratories, NI) using an automated spectrophotometer (COBAS, Roche Diagnostics Limited). The sensitivity and intra-assay coefficient of variation for this assay were 3.44 U.L⁻¹ and 1.59%, respectively.

All standards and samples were analysed in duplicate.

3.10.3 Hormones

Endocrine status was measured using selected hormones in serum in studies 2, 3 and in plasma in Study 5. Cortisol and IGF-1 were measured by commercially available enzyme immunoassays (Diagnostic Systems Laboratories Inc., Webster, Texas, USA). The manufacturer's reported sensitivity and intra-assay coefficient of variance were $0.1 \mu\text{g.dL}^{-1}$ and 5.9% for cortisol, and 0.01 ng.mL^{-1} and 6.5% for IGF-1. Cortisol was converted from $\mu\text{g.dL}^{-1}$ to nmol.L^{-1} using a conversion factor of 27.589. Combined intra- and inter-assay coefficient of variance calculated from study assay data for cortisol and IGF-1 were 1.02% and 2.56%, respectively.

Concentrations of total testosterone were also measured by commercially available enzyme immunoassays (R&D Systems Inc., Abingdon, UK). The manufacturer's reported sensitivity and intra-assay coefficient of variance were 0.030 ng.mL^{-1} and 3.3%. Combined intra- and inter-assay coefficient of variance calculated from study assay data for testosterone was 1.35%.

All standards and samples were analysed in duplicate.

3.10.4 Full blood count

A full blood count analysis was also completed in studies 2 and 3 by a haematological diagnostic system (ADVIA 2120, Siemens, Germany) measuring haemoglobin, white blood cell (WBC) count, platelet count, red blood cell (RBC) count, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and neutrophil, lymphocyte, monocyte, eosinophil and basophil populations.

3.10.5 Systemic intramuscular proteins and enzymes

In Study 3, concentrations of an intramuscular protein (myoglobin) and enzyme (creatine kinase) were measured in serum. Given that these are contained within muscle, their presence in the circulation (via leakage from damaged muscle cells) has been proposed to indicate exercise-induced muscle damage. The

relevance of creatine kinase as an indicator of muscle damage, however, has been debated. Owing to the rapidity of clearance from the circulation, it is argued that creatine kinase does not accurately indicate the presence or severity of muscle damage and that interpretation must be exercised with caution.

3.11 Injury data

3.11.1 Injury

For study 6, injury data were obtained from trainee medical records by military medical practitioners. This was completed retrospectively. Only injuries to the lower limb and lumbar spine were collected. An injury was defined as physical damage to the body that resulted in the trainee seeking medical attention (Knapik et al. 2002). A time-loss injury was defined as when an individual was assigned one or more days of limited duty as a result of the injury. Injuries were assigned to classifications of either “acute” or “overuse”. Acute injuries were those caused by a single abrupt excessive overload of the tissue or joint. Overuse injuries were those deemed to be caused by cumulative damage from continual overloading of the tissue or joint. Injuries were split into training-related and non-training-related injuries based on medical notes. This allowed the primary outcomes measures of “all injuries”, “training-related injuries” and “time-loss training injuries”. Injury type and anatomical location were also assigned. Injury classification information is displayed in Table 3.1. Specific criteria of types and sites were used to identify injuries that be categorised as medial-tibial stress syndrome (MTSS) and knee pain, which typically affect military populations. Knee pain was a grouping of all muscle, tendon cartilage, ligament and other soft tissue overuse injuries to the knee, while MTSS encompassed stress fracture, muscle strain, tendon, non-fracture bone and soft tissue overuse injuries to the tibia/fibula. Recurrent injuries were defined as injuries to the same trainee with the same cause, injury type, anatomical site and side of body to a previously sustained injury.

Injury outcome was split into one of four categories; (i) full return to training, (ii) return to training with further injury, (iii) medical discharge and (iv) non-medical discharge. The term “further injury” denoted that another injury of

any kind occurred after the injury in question. These subsequent injuries were then marked with an injury outcome. Severity of injury was measured in training time lost per injury. Training time lost was calculated by the number of days between first presentation of the injury and the date of outcome.

3.11.2 Training exposure

Information on training duration, specifically dates of entry and exit were compiled by military staff from British Army databases. Total time in training at ITC(C) was calculated by the number of days between the date of entry and the date of either completion of training or discharge from training, then subtracting the training time lost from injury. For individuals with multiple injuries, the sum of the training days lost for each injury was used. The sum of each of these gave total training exposure in trainee-days.

Table 3.1. Injury type criteria

Training Injury	Cause	Injury Type	Side	Anatomical Site
Yes	Acute	Fracture	Left	Lumbar Spine
No	Overuse	Stress Fracture	Right	Pelvis
		Muscle Strain	Bilateral	Thigh
		Bruising		Knee
		Tendon		Tibia/ Fibula
		Cartilage		Ankle
		Ligament		Foot
		Laceration		
		Blister		
		NFCI		
		Non Fracture Bone		
		Non Specific Soft Tissue		

3.11.3 Incidence of injury and injury type

Injury incidence (I) gives the average risk of sustaining one or more injuries per trainee in the duration of training. This was calculated by the number of trainees injured divided by the total number of trainees at risk. For injury incidence, 95% confidence intervals (CI) were calculated using the following formula:

$$CI = I \pm (1.96 \times SE)$$

Where I is incidence and SE is standard error.

For incidence, standard error was calculated with the following formula:

$$SE = \sqrt{\frac{I(1-I)}{n}}$$

Where n is the sample size.

Injury proportion was also calculated for injury type, by taking the number of instances of a particular type of injury and dividing by the total number of injuries. This was used to establish the proportion of total injuries that each injury type and site represented.

3.11.4 Relative risk and odds ratio

In order to compare injury incidence between groups, relative risk (RR) and odds ratio (OR) were calculated from injury incidence in each injury classification with non-smokers acting as a control group (CON). The appropriate values for each smoking group were substituted as the exposed group (EXP) in the following formulae:

$$RR = \frac{I_{EXP}}{I_{CON}}$$

Where I_{EXP} and I_{CON} are injury incidence in the exposed and control groups, respectively.

Odds ratio (OR) was calculated by the following formula:

$$OR = \frac{Injured_{EXP} \times Not\ injured_{CON}}{Injured_{CON} \times Not\ injured_{EXP}}$$

Where *Injured* is the number injured in the specified group.

To ensure the lower limits of standard error did not contain negative values, for both risk and odds ratio, data were log transformed. Positive and negative 95% confidence limits were calculated on the logarithmic scale and reverse transformed (formulae appended (C)). Relative risk and odds ratio were calculated for training-related injuries in all trainees as well as in smokers and non-smokers.

3.11.5 Clinical incidence and incidence rate

As injury incidence does not take into account multiple injuries to the same trainee, clinical incidence was also calculated. Clinical incidence is the number of injuries sustained per trainee. With each injury treated as a separate case, this was calculated by the total number of injuries divided by the number of trainees at risk. Injury incidence rate was also calculated, which is the incidence of an injury per training unit time. This allows the indication of the average number of injuries that would be sustained by a trainee depending on duration of training. This was calculated by the total number of injures divided by the exposure time (See “training exposure”) and presented as injuries per 1000 trainee-days. Confidence intervals (95%) for both clinical incidence and incidence rate were calculated as for injury incidence (above), but for incidence rate the values were multiplied by 1000 to align with 1000 trainee-days. Standard error for injury incidence rate and clinical incidence were calculated as follows:

$$SE = \frac{I}{\sqrt{Number\ of\ injuries}}$$

Where *I* is either clinical incidence or incidence rate.

Injury burden is a measure of the cost in training time elicited by an injury per unit time. It was calculated by multiplying incidence rate by average (mean) severity of injury (training days lost per injury). This gives a value of training days

lost per 1000 trainee-days. Confidence intervals (95%) and standard error for burden were calculated as above by replacing incidence with burden values.

3.11.6 Rate ratio

To compare incidence rate between non-smokers and smokers, rate ratio was calculated:

$$Rate\ ratio = \left[\frac{Injuries_{EXP}/Exposure_{EXP}}{Injuries_{CON}/Exposure_{CON}} \right]$$

Where exposure is the number of trainee-days completed in that group.

Data were log transformed for standard error and confidence interval calculation, and then reverse transformed (formulae appended (C)). Burden was compared by replacing the numerator and denominator with the burden of the exposed and control group, respectively.

CHAPTER 4

Study 1

Smoking Characteristics of British Army Trainees at Entry to Initial Training

4.1 Introduction

Cigarette smoking is considered to be the greatest preventable contributor to severe illness and premature death worldwide (Fagerström 2002; World Health Organisation 2004). It is well established that smoking adversely effects long term health; increasing risk of heart disease, chronic obstructive pulmonary diseases, cancer and immunological disorders (He et al. 1999; Soporì 2002; Doll et al. 2005; Birrell et al. 2008; Taylor 2010). Additionally, smoking may influence otherwise healthy active populations by contributing to reduced physical fitness (Bernaards et al. 2003), impaired immune function (Arcavi & Benowitz 2004; Kulkarni et al. 2010) and increased risk of musculoskeletal injury (Reynolds et al. 1999; Altarac et al. 2000; Knapik, Sharp, et al. 2001; Etherington & Owen 2002). In this context, where military populations are encouraged to maintain health and high levels of physical fitness, it is particularly noteworthy that previous research has found smoking prevalence during military service is typically higher than in the general population (Hooper et al. 2008; Dunstan 2010)

The most recent national report describes smoking prevalence in Britain to be 20% following a steady decline from 1998 to 2008 (Dunstan 2010). Smoking prevalence is, however, highly variable depending on age and on socio-economic class classified by job type, being highest at 30% in both 25-34 year old males and in manual working males (Table 2.1). In military populations, smoking prevalence can vary between trainees and individuals in active service, as well as by country and the military organisation (Table 2.3). In three studies, military trainees during basic training have compared more favourably with the general public reporting smoking prevalence as low as 6.3% and 22% in United States (US) Air force trainees (Chisick et al. 1998; Sherrill-Mittleman et al. 2009) and 24% in Norwegian basic training (Heir & Eide 1997). Contrary to this, Altarac et al. (2000) and Klesges et al. (2001) observed higher smoking prevalence in US Army trainees than values reported in service (Chisick et al. 1998; Rae Olmsted et al. 2011). Despite considerable variation between individual study populations, it would appear that

smoking prevalence in trainees is similar to that of active-duty personnel, though still greater than the general population (Table 2.3).

Additionally, it has remained unclear how military training environments affect smoking status. It is possible that in order to attain associated physical fitness and health requirements, partaking in military training may either act to reduce smoking prevalence or cause smoking cessation prior to entry. However, it has been observed that during military training the proportion of those who take up smoking is higher than those who quit (Schei & Sjøgaard 1994). As such, the military training environment may act to promote tobacco use (Nelson et al. 2009).

Previous studies describing smoking characteristics have often made the classification of smokers limited by questioning solely current smoking status (“Yes” or “No”) and average frequency (cigarettes per day). The distinct classification of current regular smokers, from former smokers and non-smokers has only been made in a handful of studies. These distinctions and demonstrating the severity of smoking exposure may aid studies in estimating the potential health risks of habitual smokers during military training. At present however, very little information is known about the smoking characteristics of trainees prior to joining active service in the British Army. Indeed, the smoking behaviour of trainees at the largest training facility in the British Army, Infantry Training Centre, Catterick (ITC(C)), has not been investigated. As such, the aim of this study was to describe smoking prevalence at ITC(C) and to give a more comprehensive understanding of the smoking behaviour of the British Army trainee population.

4.2 Methods

4.2.1 Participants

Participants in this study were a cohort of male infantry trainees (n=2087) aged between 18 and 33 years old.

4.2.2 Study design

The study contained two phases. First, the military pre-training questionnaire (MPQ) was administered to trainees in week 1 of training. Then, as a follow up phase, a shortened version of the MPQ solely containing items related to smoking was administered during medical examinations on exit from the course. Therefore, trainees who completed the follow up questionnaire will have been in one of the following three categories (i) at the completion of training, (ii) those discharged as of right or (iii) those being discharged for medical reasons. Of those who completed the questionnaire 472 trainees matched those who completed the first phase of the study and could be included in the follow up analysis

4.2.3 Data analysis

Analysis of questionnaire responses was completed on SPSS for Windows version 16.0. Percentage prevalence of current smokers, former smokers and non-smokers are presented. Mean number of cigarettes smoked per day and years smoked were calculated for regular smokers and ex-smokers. Smoking exposure in pack years was calculated by cigarettes smoked daily divided by 20 (1 pack), multiplied by years smoked. Continuous data or numerical responses to questionnaire items are presented as mean (\pm SD). For written answers or discrete data the mode is presented. Those who failed to answer all appropriate questions or gave conflicting answers were deemed non-responders and comprised 1.4% of participants.

4.3 Results

4.3.1 Smoking Prevalence in British Army Trainee Training

At entry to training, the smoking prevalence of British Army trainees whether occasionally or regularly was 53%. Of those, 91% were regular smokers (> 1 cigarette per day). Therefore, prevalence of current regular smokers was 48%.

Non-smokers and former smokers comprised 37% and 9% of trainees in the study, respectively. Of those who had ever tried smoking a cigarette, the average age for the first cigarette was 13 (± 6) years old.

4.3.2 Smoking exposure of regular smokers

The mean (\pm SD) number of years smoked by regular smokers was 6.0 (± 3.3) years. The average number of cigarettes smoked per day was 11.5 (± 5.8), with the most common responses being 10, 15 and 20 cigarettes per day reported by 32%, 20% and 11% of smokers, respectively. This gives an average smoking exposure of 3.4 pack years.

4.3.3 Characteristics of former smokers

The mean (\pm SD) number of years smoked by ex-smokers was 4.3 (± 3.1) years. Average cigarette consumption per day was 10.6 (± 5.9) with the most common response being 10 cigarettes, given by 37% of ex-smokers. The average age for starting smoking regularly was 14.5 (± 2.4) years old. Fifty two percent of former smokers stopped smoking more than a year prior to commencing military training, while 48% stopped during the year prior to commencing training.

4.3.4 Follow-up

Follow up data (n=472) showed that over the course of training 398 trainees (84%) remained the same smoking status. In this subsample of 472 trainees at entry and exit, respectively, the number of non-smokers was 159 (33.7%) and 141 (29.9%), regular smokers 251 (53.2%) and 290 (61.4%), former smokers 41 (8.7%) and 22 (4.7%), and occasional smokers 21 (4.5%) and 19 (4.0%) (Figure 4.1). Five and seven regular smokers became former and occasional smokers, respectively. Twelve and six non-smokers, respectively, became regular and occasional smokers. Twenty seven former smokers became smokers, twenty five of which smoking > 1

cigarette per day. Fourteen occasional smokers became regular smokers, and three became former smokers.

In the 239 trainees that smoked regularly at both entry and follow up, average years smoked and cigarette smoked per day at entry were 6.3 (± 3.3) and 11.7 (± 5.6), respectively. In this same sample at follow up years smoked rose to 6.5 (± 3.2) and cigarette consumption rose to 14.1 (± 5.8).

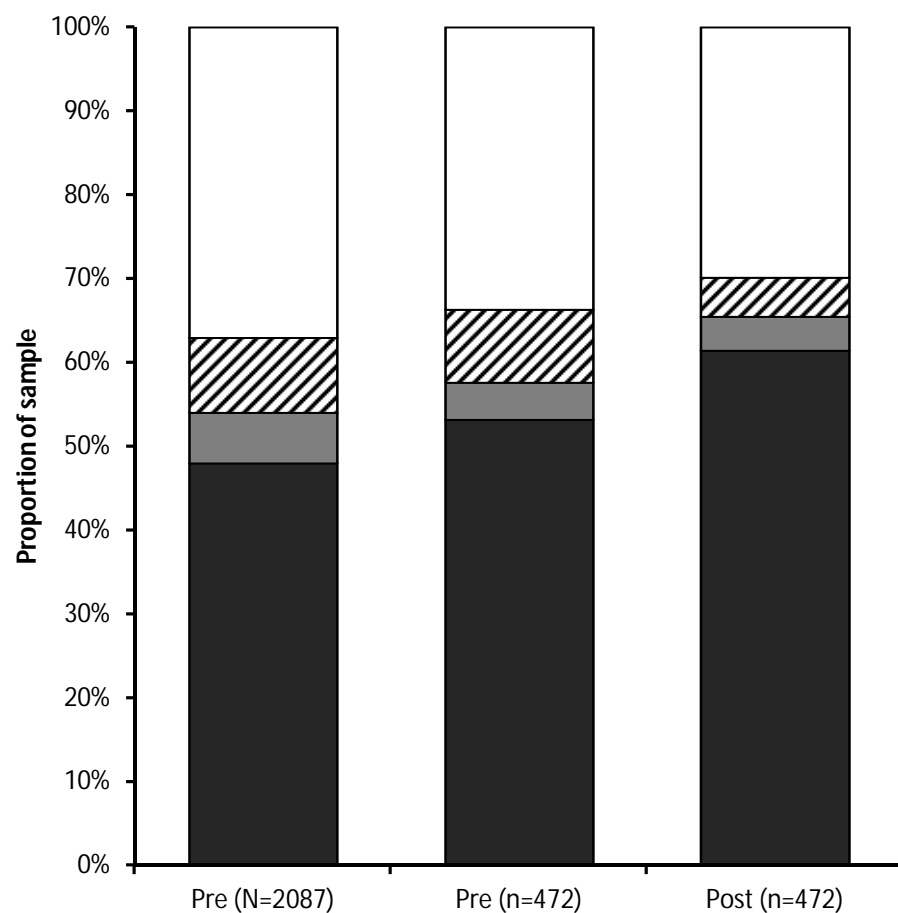


Fig 4.1. Percentage of non-smokers (white fill), former smokers (diagonal pattern fill), occasional smokers (grey fill) and habitual regular smokers (black fill) in the complete data set at entry to initial infantry training (n=2087), and in the sub-sample (n=472) at both pre training and follow up.

4.4 Discussion

In male trainees at entry to British Army training, smoking prevalence was 53% compared to 20% in the British general public (Dunstan 2010). This supports the predominance of research which suggests smoking is more prevalent in those at entry to the military than general populations. In regular smokers the mean and mode frequency of 11.5 and 10 cigarettes per day, respectively, shows cigarette consumption was similar to the national average of 10. Data from the current study suggest that over half of those entering into British Army training at ITC(C) are smokers and approximately 60% either currently smoke or have smoked regularly before. Follow up data suggest smoking consumption rate in regular smokers increased during training.

The overall prevalence of current regular smokers was markedly higher than rates recently reported in military service of ~30% (Hooper et al. 2008; Fear et al. 2010). This does not agree with the general consensus of research, typically observing smoking prevalence in training to be similar to those in military service (Klesges et al. 2001; Rae Olmsted et al. 2011). Even with a high smoking prevalence of 51% observed in Norwegian trainees (Heir & Eide 1997), the same smoking prevalence was reported in a comparable population of active Norwegian Army personnel (Schei & Sjøgaard 1994). One study found that, when comparing smoking behaviours of trainees and servicemen across all branches of the US Military, smoking prevalence was higher in enlisted men than during training in all military services (Chisick et al. 1998). It should be noted, however, that in contrast to the British military, at the time of the study and subsequently thereafter smoking was not permitted during US basic training which may have altered smoking behaviour and reduced smoking prevalence prior to enlistment.

In contrast to overall prevalence, the average cigarette consumption in trainees at ITC(C) was lower than the 15 cigarettes per day average recently reported in active duty personnel (Fear et al. 2010). In reality, however, the rates reported by Fear et al. (2010) may be positively biased by the study containing personnel during wartime deployment, which has been shown to markedly increase cigarette consumption rate (Boos & Croft 2004). Additionally, some of the variation in self-reported smoking behaviour between the current study and

previous research may be due to differences in measures of anonymity and the personnel administering questionnaires. Where some studies have used civilian researchers (Fear et al. 2010; Rae Olmsted et al. 2011) and others military staff (Klesges et al. 2001; Hooper et al. 2008), it may be that there is pressure to alter questionnaire responses from true smoking behaviour. The current study employed methods to ensure anonymity but used military staff to administer questionnaires, which may be improved by using solely research staff. Unfortunately, in research conducted on large cohorts and within military environments the limitations described above can be largely unavoidable, highlighting that drawing externally valid conclusions from smoking behaviour should be done with care.

Smoking prevalence within a population has been shown to be influenced by factors such as age and socioeconomic class which may explain the high values observed in this population. Fear et al. (2010) showed in military personnel that smoking prevalence was highest in the youngest age group (20-24 years) and in the lowest tiers of rank/socioeconomic status and education. As such, it is unsurprising that new trainees at the beginning of training, whose aims are to hold ranks below officer, would have a high smoking prevalence.

Given that previous research has shown discrepancies in smoking behaviour when comparing trainees with active duty personnel, it would be valuable to ascertain how military training itself might affect smoking prevalence or alterations in smoking habits. In the present study, follow up data showed the number of regular smokers increased by 16% during training, while all other groups reduced in size. Additionally, average smoking consumption in habitual smokers at follow-up rose by ~2 cigarettes per day, reaching similar values to those described by Fear et al. (2010). Though it would seem reasonable that the promotion of health and physical fitness in the military training environment might act to reduce smoking prevalence, other factors such as stress relief, camaraderie and easy access to cheap tobacco products may promote both the commencement and maintenance of tobacco use (Nelson et al. 2009). A study by Ebbert et al. (2006) suggested that previous non-smoking individuals often initiated smoking upon entering the military. Additionally, it has been observed that marketing strategies may directly encourage smoking behaviour in this population (Joseph, Muggli, et al. 2005). In agreement with the current study, a large cohort of Norwegian conscripts reported

55.7% of smokers increasing their smoking behaviour during military service (Schei & Sjøgaard 1994). This supports occasional and former smokers increasing or recommencing cigarette consumption as observed in the present study. Ultimately however, data from the present study indicate that although regular smoking prevalence increased, the majority of those partaking in British infantry initial training did not alter smoking status, with 84% of trainees remaining in the same smoking classification. This suggests military training may be most influential on the habits of those already smoking habitually.

This study aimed to describe the current smoking behaviour of British Army trainees entering ITC(C). The strategies used in the study for maintaining security and anonymity, and the prior testing of the questionnaire for reliability and validity provides confidence that this data accurately represent the smoking behaviour of this population. Smoking prevalence is higher than the British general public and of those previously described in similar military populations. Despite their youth, data from this study provides evidence that in some cases the cumulative smoking exposure in military trainees may be substantial. Follow up data shows that although regular smoking prevalence increased, undertaking military training did not markedly affect smoking status for the majority of trainees. However, the military training environment may have some effect on encouraging regular smokers to increase daily consumption, and for occasional and former smokers to increase or recommence regular smoking.

CHAPTER 5

Study 2

The Influence of Smoking Status on Markers of Oxidative Stress, Inflammation and Hormone Concentrations at Entry to British Army Infantry Training

5.1 Introduction

It is well established that smoking has adverse effects on health and can limit physical fitness. The development of health and physical fitness of trainees undertaking initial military training is imperative for effective entry into the armed forces. Despite this, regular smoking prevalence in a cohort of trainees in the British infantry (48%; Chapter 4) is higher than in the general population (29% males in 20-24 year age group in UK (Robinson & Bugler 2008) and reported rates for those already in service (30%; Fear et al. 2010). Interestingly, in military training where there is a high incidence of injury- and physical performance- related drop out (Blacker et al. 2005), smoking has been the most widely identified independent risk factor for injury (Reynolds et al. 1999; Altarac et al. 2000; Knapik, Sharp, et al. 2001; Etherington & Owen 2002) and linked to poorer training outcomes (Marti et al. 1988). However, the difference between the physiological state of smokers and their non-smoking counterparts in this population has not been examined. Although there is evidence that smoking could influence health and training outcomes even in a young, relatively active population, the mechanisms responsible remain under-researched.

Chronic cigarette smoking is influential in the development of many chronic diseases (Cross et al. 1998; Tanriverdi et al. 2006) as well as impaired wound healing (Sherwin & Gastwirth 1990) and increased risk of infection (Arcavi & Benowitz 2004). The possible pathogenesis of such disorders has been attributed to the physiological status of smokers when compared to non-smokers, typically characterised by chronic elevations in oxidative stress (Reilly et al. 1996) and systemic inflammation (Andelid et al. 2007) and alterations in endocrine (Steptoe & Ussher 2006) and immune function (Kulkarni et al. 2010).

Free radicals, produced in the constituents of cigarettes and tobacco smoke, induce oxidative stress in smokers (Pryor 1997) and have been associated in the

chronic smoking-induced rise in systemic inflammation. Urinary markers of oxidative stress increase in response to cigarette smoking (Pilz et al. 2000; Helmersson et al. 2005; Basu et al. 2009) and are elevated at rest in habitual smokers (Reilly et al. 1996; Helmersson et al. 2005). Chronic low-grade inflammation, indicated by persistent increases in serum interleukin (IL) -6 and the acute phase protein CRP (C-Reactive Protein), has been reported in middle aged long term smokers (Bazzano et al. 2003; Jang et al. 2007; Levitzky et al. 2008) and even in a young, otherwise healthy smoking population (O'Loughlin et al. 2008). Importantly, while transient rises can be beneficial as part of an adaptive and/or homeostatic process, chronic elevations in both oxidative stress and IL-6 have been observed to have maladaptive effects on muscle (Andrade et al. 2001; Visser et al. 2002), protein break down (Goodman 1994; Yamada, Mishima, et al. 2006) and increase susceptibility of cells to oxidative damage (Shin et al. 2007).

Both smoking and elevated inflammatory cytokine production have also been reported to influence the secretion of various hormones. The presence of nicotinic binding sites in the hypothalamus (Kellar et al. 1999) has been implicated as a mechanism by which smoking, via corticotrophin releasing hormone and adrenocorticotrophic hormone, might result in increased cortisol secretion from the adrenal gland (Steptoe & Ussher 2006). Insulin-like growth factor (IGF) -1 and its associated binding proteins are reduced in habitual smokers and correlate with cigarettes smoked daily (Renehan et al. 2004). Increases in circulating IL-6 concentrations have also been associated with reduced circulating levels of IGF-1 (De Benedetti et al. 1997; Joseph, Kenny, et al. 2005) and increased circulating cortisol concentrations (Steensberg et al. 2003). Research into the effect of smoking on testosterone has demonstrated increased, decreased or unchanged concentrations when compared to those in non-smokers (Field et al. 1994; Harman et al. 2001; Svartberg et al. 2003; Richthoff et al. 2008). Some authors have hypothesised that basal concentrations of circulating hormones, by contributing to the mediation of physiological and metabolic processes, may have implications on long-term health, growth and development, and physical recovery during consecutive days of exercise training (Kraemer et al. 1998; Kraemer et al. 1999; Nindl, Barnes, et al. 2007; Nindl et al. 2011).

Research into the adverse effects of smoking on health has predominantly studied long term smokers (≥ 10 years of smoking) who were middle-aged or older (≥ 40 years of age). In the context of the present study, the military trainee population is much younger. However, a large proportion of the British Army training population could have accumulated close to 6 years of regular tobacco exposure prior to training (Chapter 4). As such, it is possible that poorer training outcomes in smokers during military training are a result of an impaired physiological adaptation to exercise training possibly mediated by differences in basal oxidative stress, and inflammatory and endocrine status. Additionally, physiological responses may be proportional to the magnitude of smoking exposure.

Therefore, within a sample of trainees at entry to British Army infantry training we investigated the hypothesis that smokers would exhibit increased markers of oxidative stress and systemic inflammation, and altered hormone concentrations compared with non-smokers. Additionally, it was anticipated that a dose-response relationship would exist whereby subgroups with greater cigarette consumption would exhibit greater differences from non-smokers in these markers.

5.2 Methods

5.2.1 Participants

One hundred and ten male British Army line infantry trainees (age 20.2 ± 3 yr; mass 74.1 ± 9.8 kg, height 1.78 ± 0.07 m) gave written informed consent to take part in the study. Trainees were all from platoons from the same divisional company.

5.2.2 Study design

This study is a single time-point independent group comparison. In week 1 of training, resting blood samples, anthropometric data and smoking status (via lifestyle questionnaire) were collected. Blood samples were taken upon waking

(0500-0600) and participants abstained from smoking or consuming food or drink in the morning until after blood samples had been collected. On the evening of the same day as blood sample collection, body mass, height, waist circumference, estimated body fat percentage and lung function were measured.

5.2.3 Blood chemistry analysis

Serum was analysed for concentrations of malondialdehyde (MDA), lipid hydroperoxides (LOOH), CRP, IL-6, testosterone, IGF-1, cortisol and alanine transaminase (ALT) activity. A full blood count was also completed. Blood biochemistry was compared between non-smokers and current smokers as well as subgroups of current smokers based on reported number of cigarettes smoked per day (light, moderate and heavy smokers).

5.2.4 Statistical analysis

Power calculations were performed using G*Power 3.0. A medium effect size ($g=0.5$) was expected between groups; meaning a sample size of 104 was required in order to achieve a statistical power of 0.8. Effect sizes were calculated for biochemical variables in smoking/non-smoker comparisons using the Hedge's G (g) pooled standard deviation method, where small, medium and large effect sizes are defined as 0.3, 0.5 and 0.8, respectively. Statistical analyses were performed using SPSS software (SPSS for Windows: Version 16.0). Normality tests (Shapiro Wilk) were performed on all biochemical and blood count variables to determine whether data were normally distributed. To detect statistical differences between smokers and non-smokers, independent t-tests were performed in cases when data were normally distributed; and independent samples Kruskal-Wallis tests when data were non-normally distributed. To compare the four independent smoking subgroups (NS, LS, MS and HS) a one-way unpaired analysis of variance (ANOVA) was performed on all biochemical, anthropometric and lung function data to determine statistical differences between groups. It is established that when analysing physiological data an ANOVA is robust to skews to the mean (Maxwell

& Delaney, 1990) so normality test results were disregarded for subgroup analysis. Post-hoc analysis with Ryan-Holm Bonferroni Stepwise adjustment (Atkinson 2002) was used to determine the location of variance in the event of a significant effect of smoking status. An alpha level of $p \leq 0.05$ was used to define the statistical significance of within- or between- participant effects. Greenhouse Geisser ANOVA outputs were used in cases where Mauchley's test of sphericity showed data to be aspherical. Population characteristics are presented as mean \pm SD. Data analysed by independent t-test and ANOVA are presented as mean \pm SE. Data analysed by Kruskal-Wallis tests are presented as median (inter-quartile range).

5.3 Results

5.3.1 Participants

The sample was made up of 65 smokers and 45 non-smokers. Smokers consumed a mean (\pm SD) of 11.8 (\pm 5.6) cigarettes per day for an average of 6.3 (\pm 3.8) years. Of the smokers, light, moderate and heavy smoking groups comprised 17, 35 and 13 participants, respectively. Participant characteristics and anthropometric data organised by group are displayed in Table 5.1. Independent t-test results showed no significant differences ($p > 0.05$) between smokers and non-smokers in age, body mass, height, body fat percentage or waist circumference. Similarly, ANOVA showed no significant differences ($p > 0.05$) between the four smoking subgroups.

5.3.2 Oxidative stress markers: smokers versus non-smokers

Mean serum MDA was significantly higher in smokers (2.09 (\pm 0.08)) than non-smokers (1.72 (\pm 0.08)) nmol·mL⁻¹ and demonstrated a moderate effect size of smoking ($p = 0.001$; $g = 0.63$). No significant difference existed in mean serum LOOH concentrations between non-smokers (1.58 (\pm 0.08)) and smokers (1.48 (\pm 0.06)) ($p = 0.353$; $g = 0.19$) μ mol·L⁻¹.

5.3.3 Inflammatory and endocrine markers: smokers versus non-smokers

We observed no significant differences in inflammatory markers between smokers and non-smokers ($p>0.05$). Serum concentration of CRP (Median (IQR)) was $0.9 (1.3) \text{ mg}\cdot\text{L}^{-1}$ among smokers compared to $0.7 (1.3) \text{ mg}\cdot\text{L}^{-1}$ in non-smokers ($p=0.116$; $g=0.18$). Median serum concentrations of IL-6 was $1.3 (1.9) \text{ pg}\cdot\text{mL}^{-1}$ in non-smokers and $1.2 (1.3) \text{ pg}\cdot\text{mL}^{-1}$ in smokers ($p=0.556$; $g=0.29$). Median serum ALT in non-smokers and smokers was $18.0 (\pm 14) \text{ U}\cdot\text{L}^{-1}$ and $18.5 (\pm 8.3) \text{ U}\cdot\text{L}^{-1}$, respectively ($p=0.545$; $g=0.10$).

Among non-smokers, mean serum IGF-1 was $284 (\pm 10) \text{ ng}\cdot\text{mL}^{-1}$ compared to $268 (\pm 9) \text{ ng}\cdot\text{mL}^{-1}$ in smokers. Serum testosterone concentrations (Median (IQR)) in non-smokers and smokers were $10.1 (5.4)$ and $10.4 (3.7) \text{ ng}\cdot\text{mL}^{-1}$, respectively. We observed median serum cortisol of $729 (472) \text{ nmol}\cdot\text{L}^{-1}$ in non-smokers and $663 (281) \text{ nmol}\cdot\text{L}^{-1}$ in smokers. No significant differences existed between groups in IGF-1 ($p=0.276$; $g=0.22$), testosterone ($p=0.201$; $g=0.25$) or cortisol ($p=0.183$; $g=0.32$).

5.3.4 Oxidative stress markers: non-smokers versus smoking subgroups

Mean serum MDA concentrations for light ($2.09 (\pm 0.10)$), moderate ($2.05 (\pm 0.12)$) and heavy smokers ($2.18 (\pm 0.20)$) $\text{nmol}\cdot\text{mL}^{-1}$ were significantly higher than those observed in non-smokers ($1.72 (\pm 0.08)$) $\text{nmol}\cdot\text{mL}^{-1}$ ($p=0.02$; Figure 5.1: A). MDA concentrations in smoking subgroups were not significantly different from one another ($p>0.05$). There were no significant differences in serum concentrations of LOOH between non-smokers ($1.58 (\pm 0.08)$), and light ($1.46 (\pm 0.12)$), moderate ($1.51 (\pm 0.09)$) and heavy smoking groups ($1.44 (\pm 0.15)$) $\mu\text{mol}\cdot\text{L}^{-1}$ ($p=0.771$; Figure 5.1: B).

5.3.5 Inflammatory and endocrine Markers: non-smokers versus smoking subgroups

No significant differences were noted between smoking subgroups in inflammatory markers. Mean serum CRP concentrations for non-, light, moderate and heavy smokers were 1.2 (± 0.3), 1.5 (± 0.3), 1.4 (± 0.3) and 2.2 (± 1.1) $\text{mg}\cdot\text{L}^{-1}$, respectively, and are presented in panel A of Figure 5.2 ($p=0.452$). Mean serum IL-6 concentrations for non-smokers, light, moderate and heavy smokers were 1.9 (± 0.3), 1.4 (± 0.2), 1.7 (± 0.2) and 1.2 (± 0.2) $\text{pg}\cdot\text{mL}^{-1}$, respectively ($p=0.303$; Figure 4.2: B). Mean serum ALT activity was 22.3 (± 1.5), 21.2 (± 2.4), 22.5 (± 2.7) and 17.4 (± 2.5) $\text{U}\cdot\text{L}^{-1}$, for non-, light, moderate and heavy smoking groups, respectively ($p=0.581$; Figure 5.3).

Similarly, hormone concentrations did not significantly differ between subgroups. For non-smokers, light, moderate and heavy smokers mean concentrations of IGF-1 were 283 (± 10), 265 (± 21), 268 (± 11) and 272 (± 21) $\text{ng}\cdot\text{mL}^{-1}$, respectively ($p=0.746$; Figure 5.4). Mean serum concentrations of testosterone for were 9.7 (± 0.5), 9.5 (± 0.5), 10.7 (± 0.6) and 11.7 (± 0.9) $\text{ng}\cdot\text{mL}^{-1}$ for non-smokers, light, moderate and heavy smokers, respectively ($p=0.190$, Figure 5.5: A). Cortisol concentrations for non-smokers, light, moderate and heavy smokers were 787 (± 43), 616 (± 46), 729 (± 43) and 751 (± 60) $\text{nmol}\cdot\text{L}^{-1}$, respectively ($p=0.148$, Figure 5.5: B).

Table 5.1. Participant characteristics by group

Variable	Smoking Status	
	Non-smokers (n=45)	Smokers (n=65)
Age (yr)	20 ± 3	<i>LS</i> 21 ± 4
		<i>MS</i> 20 ± 3
		<i>HS</i> 20 ± 2
		<i>All</i> 20 ± 3
Body Mass (kg)	73.1 ± 11.0	<i>LS</i> 77.5 ± 10.1
		<i>MS</i> 74.5 ± 9.1
		<i>HS</i> 72.5 ± 5.8
		<i>All</i> 74.9 ± 8.9
Height (m)	1.78 ± 0.07	<i>LS</i> 1.80 ± 0.08
		<i>MS</i> 1.78 ± 0.06
		<i>HS</i> 1.77 ± 0.05
		<i>All</i> 1.78 ± 0.06
Body fat (%)	14.9 ± 3.9	<i>LS</i> 15.4 ± 2.8
		<i>MS</i> 15.7 ± 3.9
		<i>HS</i> 13.9 ± 2.5
		<i>All</i> 15.3 ± 3.4
Waist circumference (cm)	77.7 ± 6.9	<i>LS</i> 79.4 ± 7.5
		<i>MS</i> 79.6 ± 6.7
		<i>HS</i> 78.0 ± 3.9
		<i>All</i> 79.2 ± 6.4

LS (n=17): Light smokers (1-9 cigarettes per day), MS (n=35): Moderate smokers (10-19 cigarettes per day), HS (n=13): Heavy smokers (≥ 20 cigarettes per day). Data presented are mean \pm SD

5.3.6 Full blood count analysis

Owing to differences in individual sample volume, blood count data for 17 participants were incomplete and were not used in analysis. The subsequent changes in n accompany the full blood count results in Table 5.2.

Plasma white blood cell and platelet count; haemoglobin; haematocrit; and populations of neutrophils, lymphocytes, monocytes, eosinophils and basophils were similar between smokers and non-smokers ($p > 0.05$) (Table 5.2). Mean red blood cell count was significantly higher ($p = 0.04$) in non-smokers ($5.17 \pm 0.17 \cdot 10^{12} \cdot L^{-1}$) than smokers ($5.04 \pm 0.04 \cdot 10^{12} \cdot L^{-1}$). Median mean-corpuscular volume (MCV) (median (IQR)) was significantly higher ($p = 0.01$) in smokers (84.2 (5.7) fl)

compared to non-smokers (82.5 (3.7) fl) and mean average mean corpuscular haemoglobin (MCH) (mean \pm SE) was higher ($p=0.042$) among smokers (30.2 ± 0.2 pg) than non-smokers (29.7 ± 1.0 pg).

ANOVA identified a significant difference between two or more subgroups in MCV ($p=0.02$) (Table appended (D)). Post hoc analysis revealed that MCV in moderate smokers (84.7 ± 0.7 fl) was significantly higher than in non-smokers (82.5 ± 3.4 fl).

5.3.7 Lung function

One non-smoking participant was unable to take part in spirometry owing to an illness meaning the total N for lung function parameters is reduced to 109. In lung function variables independent t-tests showed no significant differences existed between non-smokers and smokers in mean (\pm SD) FEV1 (4.2 (± 0.6) vs. 4.2 (± 0.5) L), FVC (4.8 (± 0.8) vs. 4.8 (± 0.6) L) and PEF (504 (± 98) vs. 501 (± 115) L.min⁻¹) ($p>0.05$). Kruskal-Wallis test showed that median (IQR) FER was similar between non-smokers (88 (13)%) and smokers (87 (9)%) ($p>0.05$). Similarly, ANOVA indicated that no significant differences were present between smoking subgroups in lung function parameters ($p>0.05$).

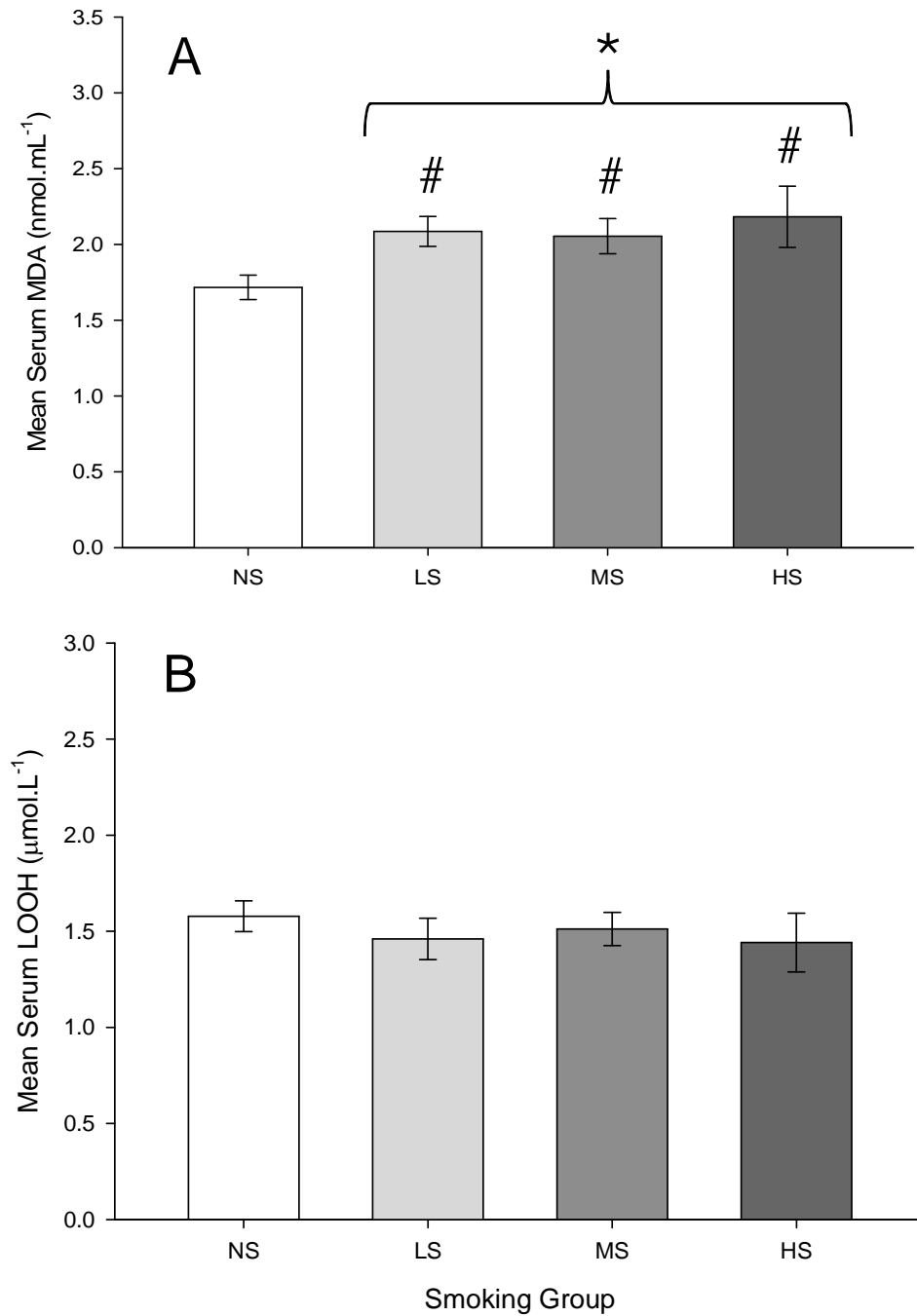


Fig 5.1. Comparison of mean serum concentrations of markers of oxidative stress MDA (A; nmol.mL⁻¹) and LOOH (B; μmol.L⁻¹) between non-, light, moderate and heavy smokers. Data are presented as means with standard error bars. *Significant effect of overall smoking status ($p=0.001$; $g=0.63$). #Significant difference between smoking subgroup and non-smoking group ($p<0.05$).

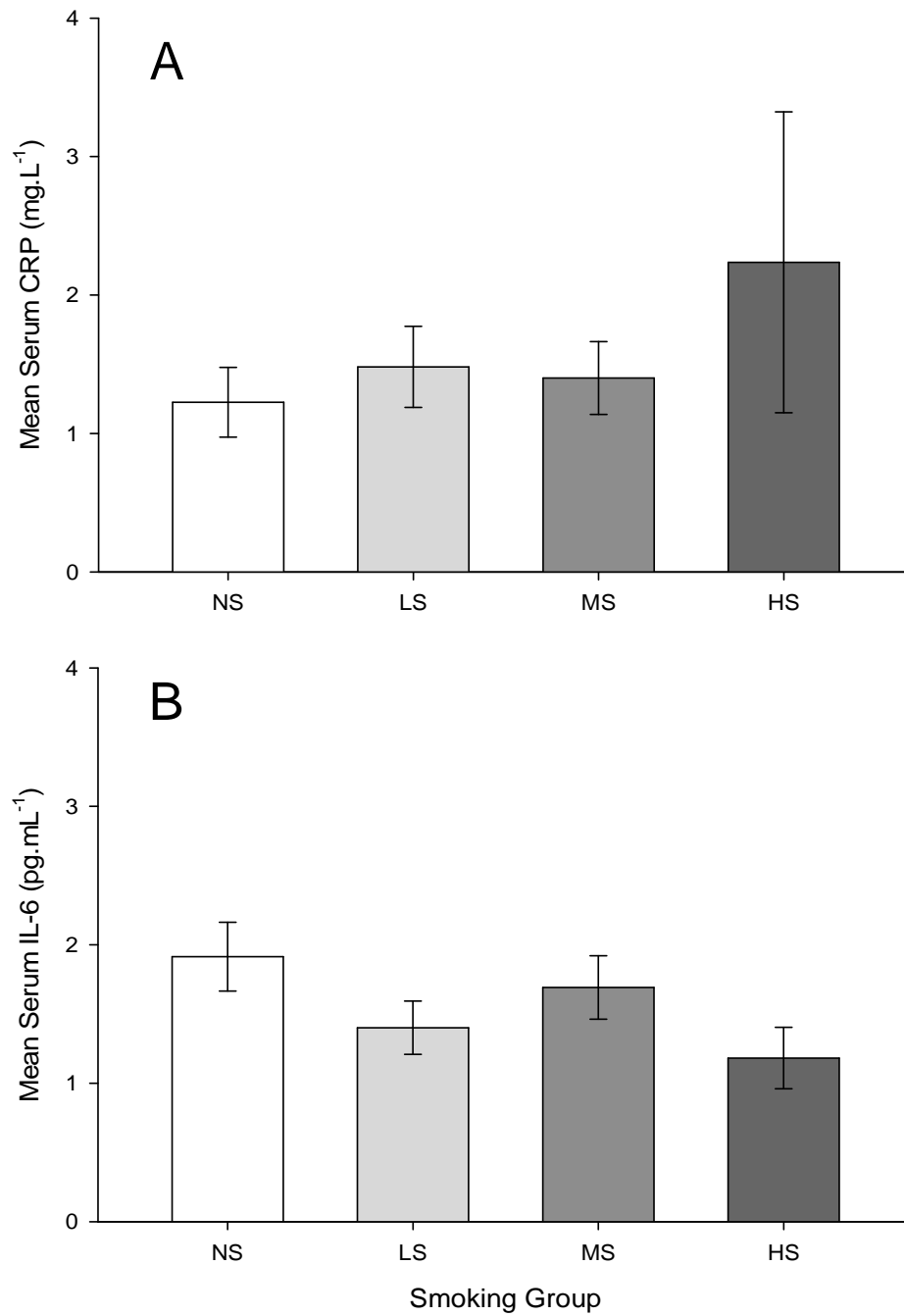


Fig 5.2. Comparison of mean serum concentrations of markers of inflammation CRP (A; mg·L⁻¹) and IL-6 (B; pg·mL⁻¹) determined by immunoassay between non-, light, moderate and heavy smokers. Data are presented as means with standard error bars. No significant differences were present between smoking subgroups in CRP ($p=0.452$) or IL-6 ($p=0.303$).

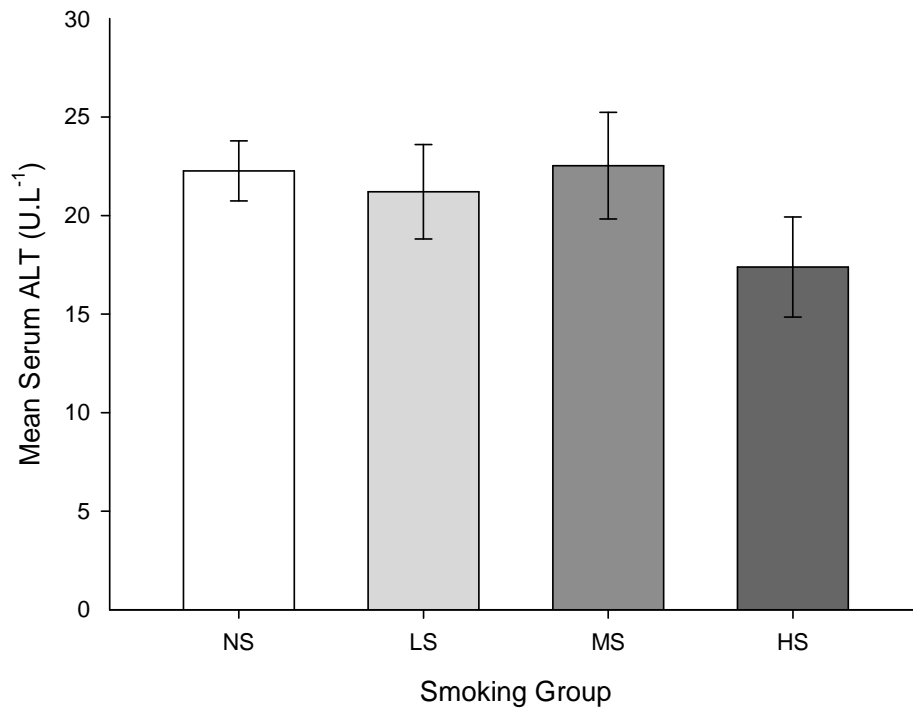


Fig 5.3. Comparison of mean serum activity of ALT (U·L⁻¹) determined between non-, light, moderate and heavy smokers. Graph displays data as group means with standard error bars. No significant differences were present between smoking groups in ALT ($p=0.581$).

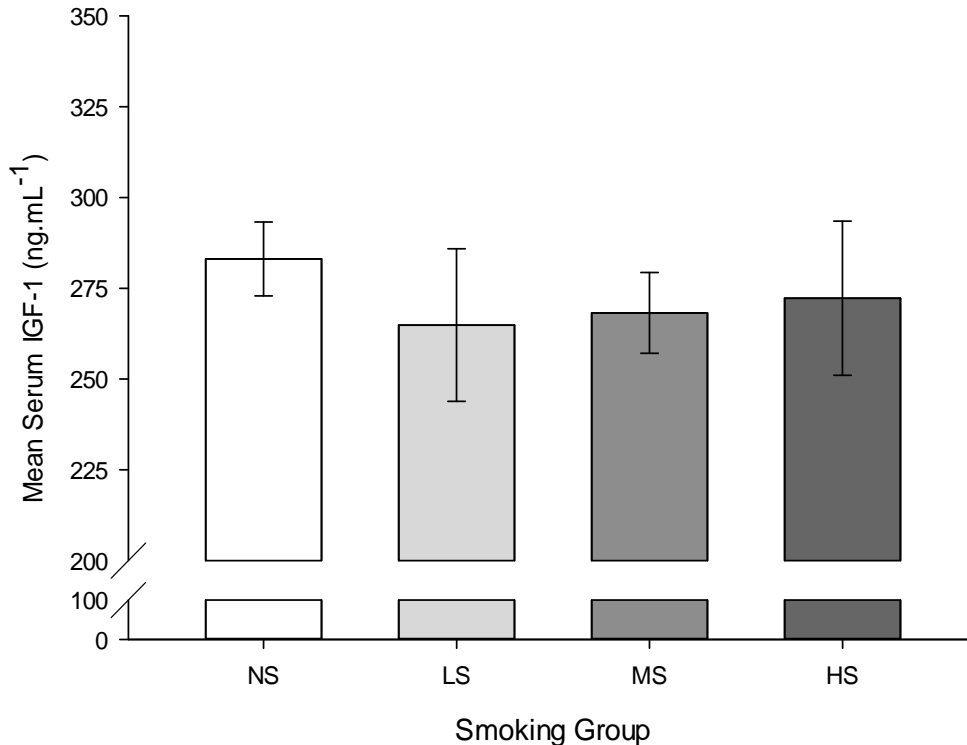


Fig 5.4. Comparison of mean serum concentration of IGF-1 (ng·mL⁻¹) determined by immunoassay between non-, light, moderate and heavy smokers. Graph displays data as group means with standard error bars. No significant differences were present between smoking groups in IGF-1 ($p=0.746$).

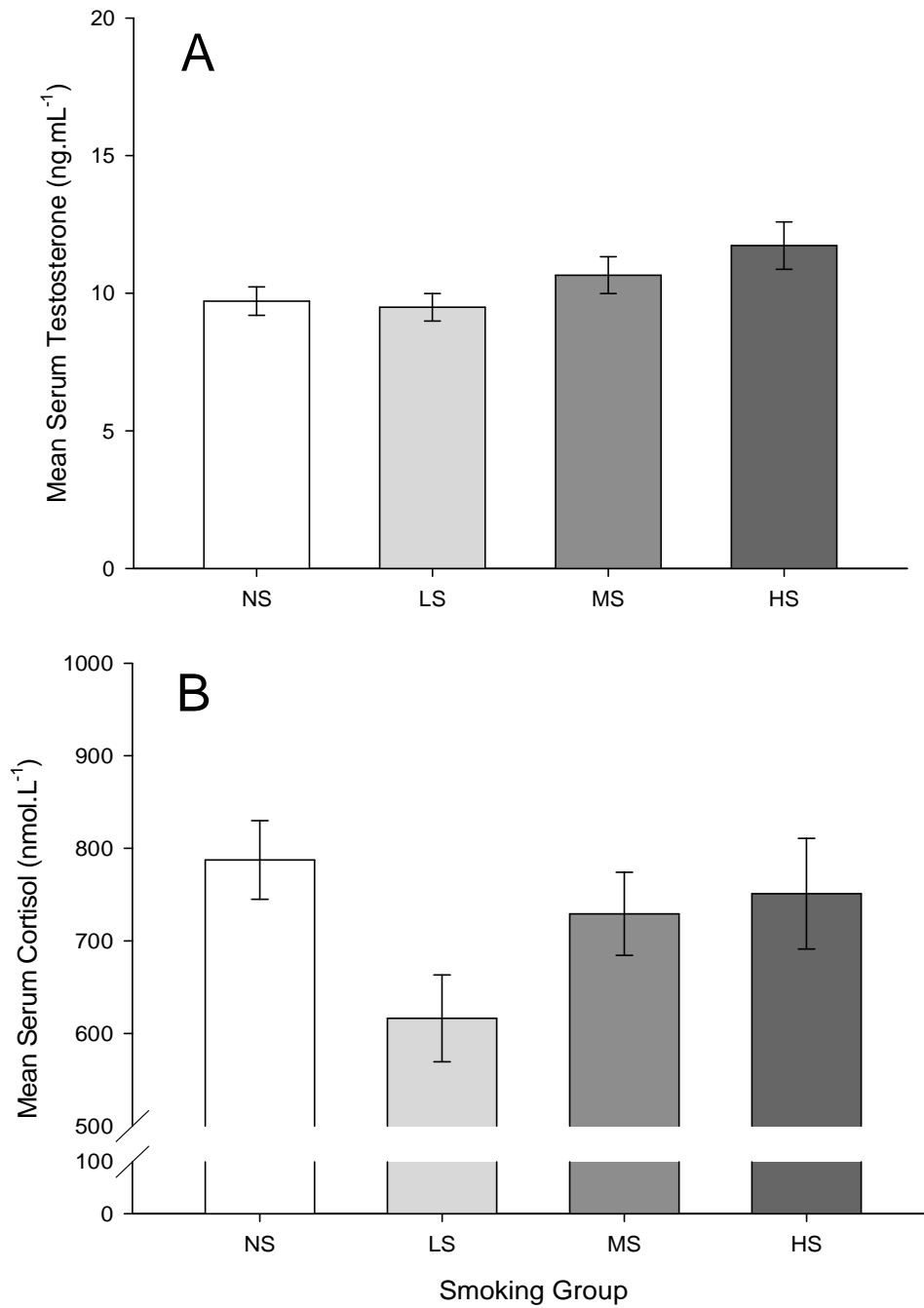


Fig 5.5. Comparison of mean serum concentration of testosterone (A; ng.mL⁻¹) and cortisol (B; nmol.L⁻¹) determined by immunoassay between non-, light, moderate and heavy smokers. Data are presented as means with standard error bars. No significant differences were present between smoking groups in testosterone ($p=0.190$) or cortisol ($p=0.148$).

Table 5.2. Full blood count data organised by smokers and non-smokers.

Variable	Measure of Central Tendency	Normative Range	Smoking Status	
			NS (n=36)	S (n=57)
(HB)				
Haemoglobin (g/dl)	Mean (SE)	13-18	15.3 (±0.2)	15.2 (± 0.1)
WBC (10 ⁹ /l)	Median (IQR)	4-11.0	7.9 (2.6)	8.3 (2.4)
(PLT) Platelets (10 ⁹ /l)	Median (IQR)	150-400	258 (68)	259 (73)
RBC (10 ¹² /l)	Mean (SE)	4.5-6.5	5.2 (±0.1)*	5.0 (± 0.0)
(HCT) haematocrit (%)	Median (IQR)	0.40-0.54	0.43 (0.03)	0.43 (0.03)
(MCV) Mean corpuscular volume (fl)	Median (IQR)	78-98	82.5 (3.7)	84.2 (5.7) †
(MCH) Mean corpuscular haemoglobin (pg)	Mean (SE)	27.5-32.5	29.7(±0.2)	30.2 (± 0.2) †
Neutrophils (10 ⁹ ·L ⁻¹)	Median (IQR)	2-7.5	3.8 (1.7)	4.1 (1.9)
Lymphocytes (10 ⁹ ·L ⁻¹)	Median (IQR)	1.5-4.0	2.8 (0.8)	2.6 (0.9)
Monocytes (10 ⁹ ·L ⁻¹)	Median (IQR)	0.2-0.8	0.7 (0.2)	0.7 (0.3)
Eosinophils (10 ⁹ ·L ⁻¹)	Median (IQR)	0.0-0.4	0.2 (0.2)	0.2 (0.2)
Basophils (%)	Median (IQR)	0.0-0.2	0.0 (1.1)	0 (1.1)

Note: Values are either Mean (Standard Error) or Median (Inter-quartile range) indicated by the “measure of central tendency” column. * indicates RBC count is significantly higher in NS than S (p=0.04). † indicates MCV and MCH is significantly higher in S than NS (p≤0.05).

5.4 Discussion

The current study made two comparisons in a sample of trainees entering infantry training in the British Army. Firstly, resting hormone concentrations and markers of inflammation and oxidative stress were examined in smokers and non-smokers. Secondly, smokers were grouped by cigarette consumption and compared to non-smokers. It was expected that oxidative stress, endocrine and inflammatory markers at entry to training would differ between smokers and non-smokers and differences would intensify with cigarette consumption, subsequently giving some indication as to the chronic effect of smoking on this population. The main finding of the study was that habitual smokers exhibited significantly higher markers of chronic oxidative stress than non-smokers, and that this was evident in each smoking subgroup. There were, however, no significant differences between smokers and non-smokers in endocrine and inflammatory markers at entry to training. Additionally, it appears no differences existed in oxidative stress or other biochemical markers as a result of greater habitual smoking exposure.

The current study showed serum concentration of oxidative stress marker malondialdehyde (MDA), but not lipid hydroperoxide (LOOH), was significantly higher in smokers and smoking sub-groups than in non-smokers ($p < 0.05$). Owing to greater stability than LOOH, MDA is typically utilised to demonstrate long term or chronic states of oxidative stress (Del Rio et al. 2005), whereas an elevation in highly-reactive hydroperoxides would indicate more recent acute oxidative processes (Davison, Hughes & Bell 2005; Fogarty et al. 2011). As such, the present study agrees with previous observations of an elevated resting state of oxidative stress in habitual smokers (Reilly et al. 1996; Tanriverdi et al. 2006; Taylor, Bruno & Traber 2008). Acute markers of oxidative stress rise as an immediate response to smoking (Morrow et al. 1995). As blood samples in the present study were taken upon waking following overnight abstinence from smoking, this is likely to explain similar LOOH levels between groups. Subgroups organised by increasing cigarette consumption exhibited similar MDA levels to one another, offering little evidence for the existence of a dose-response relationship between smoking and oxidative stress. Despite several authors reporting an association between oxidative stress, hormonal and immune-inflammatory response (Conner & Grisham 1996; van der

Vaart et al. 2004; Federico et al. 2007), the present study gives no clear indication of an effect of elevated oxidative stress on other biochemical markers in smokers.

Interleukin-6, alongside a cascade of inflammatory mediators, stimulates the production of CRP (Heinrich et al. 1990). As such, no difference in IL-6 concentration between smokers and non-smokers ($p=0.556$) is likely to be responsible for similar CRP levels between groups. This is contrary to numerous population- and laboratory- based studies demonstrating CRP (Bazzano et al. 2003; Jang et al. 2007; Sunyer et al. 2009) and IL-6 (Levitzky et al. 2008; Sunyer et al. 2009) to be significantly raised in smokers. This finding is more surprising given the elevation in oxidative stress observed in the current sample. However, research in the inflammatory response to smoking predominantly focuses on middle aged or elderly long term smokers, presenting the cumulative effect of long term smoking combined with the rise in inflammation associated with age (Ershler & Keller 2000; Krabbe et al. 2004; Dixon et al. 2009). We are only aware of one study that reported a chronic elevation in CRP in an otherwise-healthy smoking adolescent population, using a large sample population (O'Loughlin et al., 2008; $N=1501$). The current sample represents individuals with substantially lower life-time smoking exposure meaning changes in levels in inflammation may not have developed, possibly explaining the failure for smokers to present significantly elevated inflammation despite greater oxidative stress.

Smoking-induced inflammation and health outcomes have been observed to be substantially greater in heavy smoking groups (≥ 20 cigarettes per day) (Byron, Varigos & Wootton 1994; Bernaards et al. 2003). As CRP levels in smoking subgroups did not significantly differ from non-smokers ($p=0.452$), magnitude of cigarette consumption appeared to elicit no effect on increasing basal CRP. Although, it should be noted that all smoking groups, especially that of the heavy smoking group, exhibited higher CRP values than non-smokers. The small effect of smoking on CRP ($g=0.18$) and standard error observed within the heavy smoking group suggest individual variability may have reduced the ability to recognise an influence of smoking on CRP. When alanine transaminase, an enzyme associated with liver function, is elevated it is often accompanied by elevated circulatory CRP, likely due to a shared source such as liver damage. However, ALT did not differ significantly between groups ($p=0.581$) and was within normative range for healthy

individuals (Giannini, Testa & Savarino 2005), providing support that inflammatory markers in the present study were not affected by liver health.

Both IGF-1 and cortisol can be mediated by smoking-induced increases in circulating IL-6 (Lieskovska et al. 2002; Steensberg et al. 2003; Joseph, Kenny, et al. 2005) and the indirect actions of nicotine on hormone release (Kellar et al. 1999; Steptoe & Ussher 2006). In the current study, similar IL-6 levels in all groups may explain unaffected IGF-1 and cortisol in smokers, but also suggests that the effect of nicotine was negligible, possibly due to overnight smoking abstinence. Though the consensus of research suggests the presence of IGF-1 and circulating binding proteins (Kaklamani et al. 1999; Holmes et al. 2002) are reduced in smokers in a dose-response manner (Renehan et al. 2004), evidence exists in support of current findings, for no measurable difference from non-smokers (Palmer et al. 2003). The above studies examined older populations than the current study, and the relationship between IGF-1 and age has been shown to be non-linear and contain considerable variation (Renehan, O'Dwyer & Shalet 2000; Juul 2003). As such, like inflammation, if an exposure-related reduction in IGF-1 from smoking exists in some populations it may not be easily discernible given the youth and duration of smoking exposure in the current population. It is noteworthy that some authors have suggested that smoking, irrespective of circulating concentration, may still affect the function of IGF-1 in glucose homeostasis and bone metabolism (Yakar et al. 2001; Juul 2003), with possible negative implications for long-term exercise training.

Several studies have examined testosterone concentrations in smokers, with some reporting greater (Gray et al. 1991; Field et al. 1994; Svartberg et al. 2003; Svartberg & Jorde 2007), others reporting lesser (Zmuda et al. 1997), and others reporting no measureable difference when compared to non-smokers (Harman et al. 2001; Richthoff et al. 2008), in agreement with the current study. Some of the uncertainty in the area appears to be explained by assay variability and how smoking may influence bioavailability of testosterone. Theoretically, testosterone bioavailability is reduced by the presence of sex hormone binding globulin (SHBG), which although only measured in a handful of studies, is elevated in smokers (Field et al. 1994; English et al. 2001; Svartberg et al. 2003). As such, smoking may solely influence the bioavailable proportion of testosterone and not total

testosterone concentration as measured in the present study. Additionally, smoking may affect overall daily secretion of testosterone and cortisol. If this were the case, the circadian rhythm of these hormones, peaking in the early morning and reaching a nadir in the early evening, means that timing of blood sampling could be a confounding factor (Kapoor & Jones 2005).

Associations between long term smoking and haematological factors have been widely established (Corre, Lellouch & Schwartz 1971; Helman & Rubenstein 1975; Bain et al. 1992). Similar to the current study, two such studies found mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) to be elevated in smokers (Mercelina-Roumans, Ubachs & Van Wersch 1994; Kung, Wang & Tseng 2008). Interestingly, red blood cell count was significantly higher in non-smokers than smokers overall. This may suggest that smoking may decrease red blood cell count but increase the size and oxygen carrying capacity of the less abundant individual cells. Research to date has not identified the mechanisms concerned but it is possible this is an adaptation to excess carbon monoxide inhalation from smoking. Given the importance of oxygen carrying capacity of the blood on endurance performance, this may have implications on physical fitness in smokers (Bassett & Howley 2000). In contrast, lung function did not differ as a result of smoking, or greater smoking exposure. This may further indicate that typical adverse effects of smoking may not have progressed given the relative youth of sample compared to populations used in previous research.

This study was the first to measure oxidative stress and inflammatory and hormonal factors at entry to British Army infantry training and attempt to elucidate a chronic influence of smoking on this population. In agreement with previous research an indicator of chronic oxidative stress was significantly higher in smokers. However, chronic differences in inflammatory markers or hormone concentrations between smokers and non-smokers, or a dose-response to smoking were not evident. Therefore, from the current findings there is no evidence of an influence of a chronic state of oxidative stress in habitual smokers on the other processes examined. If causal mechanisms were to exist that underpin the association of smoking with injury risk and reduced physical fitness observed in epidemiological data using similar populations, they are not highlighted by the current study. Considerable variation in CRP concentrations, especially in heavy

smokers, could potentially have obscured inferential measures of the influence of smoking in this sample. Equally, given that in adolescence and young adulthood the effects of smoking could be considered reversible by cessation, as evidenced in a reduction in markers of oxidative stress following two weeks of cessation in smokers (Morrow et al. 1995), the chronic negative effects of smoking may be lessened in healthy, young individuals, only becoming evident later in life. Differences in these biochemical markers may be more discernible in the presence of training stimuli, and thereby influence injury risk and adaptation or recovery in smokers during training itself.

CHAPTER 6

Study 3

The Influence of Smoking Status on Physical Performance Adaptation and Markers of Oxidative Stress, Inflammation and Endocrine Status during 10 Weeks of Military Training

6.1 Introduction

Initial military training is an intense process of physical fitness development involving arduous and often unaccustomed exercise. In the British Army, Infantry training is reported to have the second lowest first-time pass rate (Carter et al, 2006) and the highest medical discharge rate (Blacker et al. 2008) of all training regiments. Given the high incidence of smoking in this population (48%: Chapter 4), it is particularly relevant that long term smoking prior to military training is associated with higher risk of training-related injury and poorer training outcomes (Marti et al. 1988; Reynolds et al. 1999; Altarac et al. 2000; Knapik, Sharp, et al. 2001). Evidence exists for lower physical fitness (Boyce et al. 2006) and reduced performance in strength tasks (Al-Obaiddi et al. 2004) in habitual smokers. However, it is unclear whether smoking negatively impacts the development of physical fitness. We are only aware of one study that has examined this, reporting that improvement in performance in an Army prescribed strength and endurance test after a six month physical fitness programme was significantly smaller in trainees who smoked (Hoad & Clay 1992).

Chronic disruptions of oxidative stress (Reilly et al. 1996; Cross et al. 1998; Isik et al. 2007), inflammation (Andelid et al. 2007) and hormones (Steptoe & Ussher 2006) observed in smokers might be possible mechanisms for attenuated adaptation to exercise training. At entry to British infantry training, smokers exhibited a significantly elevated resting state of oxidative stress compared to non-smokers, but levels of several endocrine and inflammatory markers were similar (Chapter 5). It is not known how these markers might alter during a period of training and how this might influence improvement in physical performance.

Smoking causes local and circulatory accumulation of oxidants, or reactive oxygen species (ROS), which can cause damage to cellular membranes (Comporti

et al. 2008) and DNA (Nair et al. 1996) by lipid and protein peroxidation. In muscle, transient production of ROS are obligatory for optimal skeletal muscle function (Reid et al. 1993) and invoke signalling pathways beneficial for adaptation and cell regeneration (Jackson et al. 2002; McArdle et al. 2002; Close et al. 2005; Jackson 2005). However, continual elevation of ROS in muscle restricts modulation of redox balance, causes oxidative damage to myosin heavy chains (Coirault et al. 2007) and can exacerbate development of muscle damage following exercise (Close et al. 2007).

Smoking-induced oxidative stress is also implicated in the chronic low-grade inflammation observed in middle-aged (Bazzano et al. 2003; Jang et al. 2007; Levitzky et al. 2008), and young, otherwise healthy smoking populations (O'Loughlin et al. 2008). Chronically elevation of the inflammatory cytokine interleukin(IL)-6 has been reported, in animal and human models, to have a maladaptive effect on muscle size (De Benedetti et al. 1997; Visser et al. 2002), protein break down (Goodman 1994; Tidball 2005) and increase susceptibility of cells to oxidative damage (Shin et al. 2007). Equally, prolonged elevated endogenous levels of C-reactive protein (CRP), stimulated by IL-6, closely correlates with loss of muscle mass (De Benedetti et al. 1997; Schaap et al. 2006) and is inversely proportional to estimated maximal oxygen uptake (Kuo et al. 2007). As such, it is proposed that a chronic elevation in these markers in a habitual smoking population might reflect disruption of pro-adaptive responses to long-term exercise training.

Total and bioavailable concentrations of hormones are also influenced by smoking and IL-6, reducing circulating insulin-like growth factor (IGF)-1 (Renehan et al. 2004; Joseph, Kenny, et al. 2005; O'Connor et al. 2008) and stimulating secretion of cortisol (Kirschbaum et al. 1992; Field et al. 1994; Steptoe & Ussher 2006; Badrick et al. 2007; Steensberg et al. 2003). IGF-1 plays a role in local signalling for hypertrophy of skeletal muscle (Adams & McCue 1998; Rommel et al. 2001; Bassel-Duby & Olson 2006) as well as glucose regulation (Yakar et al. 2001). Research into the effect of smoking on testosterone has demonstrated higher (Gray et al. 1991; Field et al. 1994; Svartberg et al. 2003), lower (Zmuda et al. 1997) and similar (Harman et al. 2001; Richthoff et al. 2008) concentrations when compared to non-smokers. Examining adaptations in these markers alongside

physical performance during military training, and identifying whether these differ between smokers and non-smokers, may help to explain any influence of smoking on the training response in this population.

The aim of this study was to investigate whether training would elicit different changes in resting markers of oxidative stress, systemic inflammation and hormone concentrations in smokers compared to non-smokers. Physical performance variables and lower leg muscle characteristics were also examined to determine whether performance improvement and adaptation differed between smokers and non-smokers across training weeks.

6.2 Methods

6.2.1 Participants

Sixty five male British Army line infantry trainees (age 21 ± 3 yr; mass 75.5 ± 8.4 kg; height 1.78 ± 0.07 m) took part in the study.

6.2.2 Study design

Smoking status was determined using lifestyle questionnaire at entry to training and at weeks 5 and 10. Blood samples were taken upon waking (0500-0600) in trainee accommodation lines early in weeks 1, 5 and 10 of training. Performance in physical tasks, lung function tests, anthropometric data and lower leg muscular characteristics were measured at weeks 1 and 10. Military fitness test performance was also collected at weeks 1 and 14. An overtraining questionnaire was also administered at weeks 1, 5 and 10 to all participants.

6.2.3 Blood chemistry analysis

Serum was analysed for concentrations of malondialdehyde (MDA), lipid hydroperoxides (LOOH), CRP, IL-6, testosterone, IGF-1, cortisol, myoglobin,

creatine kinase and alanine transaminase (ALT) activity. A full blood count was also completed. Blood biochemistry was compared between non-smokers and subgroups of current smokers based on reported number of cigarettes smoked per day (light, moderate and heavy smokers).

6.2.4 Anthropometric measurements and physical performance testing

On the evening of the same day as blood sample collection body mass, height, estimated body fat, waist circumference and lower leg muscular characteristics were measured. Maximum strength performance in chest press, seated row, leg press, static lift exercises was measured alongside jump height. Owing to injury some participants did not complete every test within each stage of physical performance data collection and were therefore excluded from the analysis for that exercise test across all weeks. The differing group n is detailed in associated results tables. These variables were compared solely between non-smokers and current regular smokers.

6.2.5 Statistical analysis

Statistical analyses were performed using SPSS software (SPSS for Windows: Version 16.0). Initially, an independent t-test was performed on baseline anthropometric data to test for any initial differences between groups. As the study design is a repeated measures independent group comparison with one unpaired (smoking) and one paired (time) independent variable all biochemical and performance variables were analysed by two-way mixed model analysis of variance (ANOVA). Post-hoc analysis with bonferroni adjustment was used to determine the location of variance in the event of a significant interaction or training effect when analysing more than two time points or groups. Tests for normality were not performed as it is established that when analysing physiological data an ANOVA is robust to skews to the mean (Maxwell & Delaney, 1990). An alpha level of $p \leq 0.05$ was used for defining the statistical significance of within- or between- participant effects. Greenhouse Geisser ANOVA outputs were used in cases where

Mauchley's test of sphericity showed data to be aspherical. Population characteristics are presented mean \pm SD. Unless otherwise stated, all data are presented as mean \pm SE.

6.3 Results

6.3.1 Participants

The sample was made up of 24 non-smokers and 41 smokers. Smoking characteristics (mean (\pm SD)) at entry to training for participants in the smoking group were an average of 12.7 (\pm 6.0) cigarettes per day for 7.2 (\pm 4.5) years (4.5 pack years). Of the smokers, light, moderate and heavy smoking groups comprised 9, 20 and 12 participants, respectively. Sufficient data for anthropometric and performance measures were present for forty six trainees (22 non-smokers, 24 smokers).

6.3.2 Anthropometric data

Body mass did not differ between non-smokers and smokers or over time ($p>0.05$). Irrespective of group, height ($p<0.001$), estimated body fat percentage ($p<0.001$) and waist circumference ($p=0.04$) significantly decreased from baseline over 10 weeks of training. No significant differences or interaction effects existed between non-smokers and smokers in any anthropometric variable (Table 6.1).

6.3.3 Physical performance data

ANOVA showed that there were no significant effects of smoking status, irrespective of time, on performance in physical tasks over 10 weeks of training ($p>0.05$; Table 6.2). Irrespective of group, performance in static lift ($p<0.001$), bench press ($p=0.004$) and leg press ($p<0.001$) improved significantly from baseline as a result of training. Additionally, ANOVA identified a significant interaction

effect in bench press performance ($p=0.031$) whereby smokers improved substantially more than non-smokers as a result of training.

6.3.4 Army physical fitness tests

Training produced a significant improvement in press up, sit up and 2.4 km run performance over 14 weeks irrespective of group ($p<0.001$). Additionally, a trend that approached significance existed in run performance, whereby improvement in non-smokers was greater than in smokers over the 14 weeks ($p=0.067$). No significant differences existed between smoking and non-smoking groups in British Army physical fitness test variables (Table 6.3).

Table 6.1. Anthropometric characteristics across training weeks organised by group (NS, $n=22$; S, $n=24$). Values are means \pm SD

Variable	Smoking Status	Week of training	
		1	10
Height (m)*	NS	1.77 ± 0.08	1.77 ± 0.08
	S	1.79 ± 0.07	1.79 ± 0.07
Mass (kg)	NS	74.9 ± 9.3	75.4 ± 8.5
	S	77.6 ± 7.3	77.1 ± 6.2
Body Fat (%)*	NS	15.7 ± 4.2	$14.7 \pm .35$
	S	15.7 ± 3.5	14.3 ± 2.6
Waist Circumference* (cm)	NS	79.3 ± 6.0	78.7 ± 4.6
	S	81.6 ± 5.9	79.3 ± 4.2

Note: * denotes a significant effect of training ($p \leq 0.05$) irrespective of group.

6.3.5 Lower leg characteristics (Peripheral Quantitative Computed Tomography)

No significant interaction or smoking status effects existed in any lower leg characteristic ($p>0.05$; Table 6.4). Between weeks 1 and 10, irrespective of group,

mean muscle area ($p<0.001$) total density of muscle and fat ($p<0.001$) significantly increased, while fat/muscle area ratio decreased ($p=0.012$). No training effects existed in fat area ($p=0.126$) or mean muscle density ($p=0.545$)

6.3.6 Lung function

Owing to illness causing several individuals to be unable to complete the tests, full data from spirometry were only available for 21 non-smokers and 24 smokers (Table 6.5). Irrespective of group, training induced a significant increase from baseline in forced expiratory ratio ($p<0.001$) and peak expiratory flow ($p=0.035$), but a decrease in forced vital capacity ($p<0.001$). Forced expiratory ratio was significantly higher in non-smokers than smokers ($p=0.029$). No interaction effects were found in any lung function variable over 10 weeks of training.

6.3.7 Overtraining

Mean (\pm SD) scores on the overtraining questionnaire for weeks 1, 5 and 10 for non-smokers were 7.1 (± 6.6), 12.8 (± 9.6) and 10.7 (± 8.3) respectively, and 6.9 (± 5.8), 14.7 (± 8.0) and 12.3 (± 9.4), respectively for smokers. At no point did group averages fall within the range of indicating overtraining. ANOVA indicated that irrespective of group a training effect existed ($p<0.001$). Post hoc analysis showed that all time points were significantly distinct from one another whereby week 10 values were higher than week 1, and week 5 significantly higher than week 10. No smoking status or interaction effects existed ($p>0.05$). Cumulative symptoms of overtraining were more common in non-smokers than smokers across the 10 weeks of training.

6.3.8 Oxidative stress markers

Serum MDA was significantly higher in smokers ($p=0.026$) than non-smokers, independent of time (Figure 6.1; Panel A). In subgroup analysis of MDA

concentrations, no significant interaction or group effects existed ($p>0.05$) at weeks 1, 5 and 10 between non-smokers, light, moderate or heavy smokers (Figure 6.1; Panel B). In both analyses values in week 10 were significantly lower than week 1 ($p<0.05$), irrespective of group.

Serum LOOH at weeks 1, 5 and 10 for non-smokers ($1.70 (\pm 0.14)$; $1.53 (\pm 0.13)$; $1.56 (\pm 0.11) \mu\text{mol}\cdot\text{L}^{-1}$) and smokers ($1.50 (\pm 0.09)$; $1.43 (\pm 0.08)$; $1.38 (0.08) \mu\text{mol}\cdot\text{L}^{-1}$) were not significantly different irrespective of time, and no interaction or training effects existed ($p>0.05$). In subgroup analysis, LOOH at weeks 1, 5 and 10 contained no significant group, interaction or training effects ($p>0.05$; Figure 6.2).

6.3.9 Inflammatory markers

Independent of time, serum CRP concentrations at weeks 1, 5 and 10 were significantly higher in smokers ($1.80 (\pm 0.38)$; $3.44 (\pm 0.60)$; $2.93 (\pm 0.55) \text{mg}\cdot\text{L}^{-1}$) than non-smokers ($1.51 (\pm 0.43)$; $2.22 (\pm 0.45)$; $1.49 (\pm 0.30) \text{mg}\cdot\text{L}^{-1}$) ($p=0.047$; Figure 6.3). In both groups serum CRP peaked in week 5 before declining by week 10, but only non-smokers returned to near pre-training levels. Serum CRP concentrations in subgroups did not significantly differ as a result of smoking status ($p>0.05$; Figure 6.3). In both analyses of CRP no interaction or training effects existed ($p>0.05$).

No significant group, interaction or training effects existed in serum IL-6 concentrations when comparing non-smokers ($1.85 (\pm 0.33)$; $1.82 (\pm 0.41)$; $1.41 (\pm 0.20) \text{pg}\cdot\text{mL}^{-1}$) and smokers ($1.44 (\pm 0.15)$; $1.97 (\pm 0.30)$; $1.79 (\pm 0.26) \text{pg}\cdot\text{mL}^{-1}$), or between smoking subgroups ($p>0.05$; Figure 6.4).

Serum ALT activity for weeks 1, 5 and 10 for non-smokers ($26.5 (\pm 2.5)$; $34.0 (\pm 3.8)$; $26.3 (\pm 2.2) \text{U}\cdot\text{L}^{-1}$) and smokers ($19.7 (\pm 1.4)$; $28.7 (\pm 1.6)$; $24.4 (\pm 2.4) \text{U}\cdot\text{L}^{-1}$) did not significantly differ irrespective of time ($p>0.05$). Similarly, subgroup analysis reported no significant interaction or group effects ($p>0.05$). In both analyses a significant training effect existed whereby week 5 values were significantly different from those at weeks 1 and 10 ($p<0.05$; Figure 6.5).

Table 6.2. Physical performance characteristics across training weeks organised by group. Values are means \pm SD.

Variable Type	Variable	Smoking Status (n)	Week of training	
			1	10
Max Strength	Static Lift (kg)*	NS (22)	149.4 \pm 24.5	159.8 \pm 28.2
		S (24)	154.1 \pm 23.8	169.7 \pm 31.8
	Bench Press (kg)*†	NS (22)	72.2 \pm 12.3	72.8 \pm 12.9
		S (24)	69.1 \pm 8.9	72.8 \pm 6.8
	Leg Press (kg)*	NS (21)	181.9 \pm 28.2	201.3 \pm 29.0
		S (24)	189.5 \pm 30.0	209.8 \pm 22.6
	Seated Row (kg)	NS (22)	70.9 \pm 8.4	70.1 \pm 9.1
		S (24)	71.1 \pm 7.2	71.7 \pm 6.3
	Grip Right (kg)	NS (22)	48.0 \pm 5.2	48.3 \pm 4.8
		S (23)	48.0 \pm 5.2	50.1 \pm 5.5
	Grip Left (kg)	NS (22)	47.8 \pm 7.1	47.0 \pm 6.2
		S (24)	48.1 \pm 6.8	49.3 \pm 5.5
Explosive power	Jump (cm)	NS (21)	33.5 \pm 5.4	35.1 \pm 6.3
		S (24)	34.4 \pm 4.6	33.8 \pm 3.6

Note: *denotes a significant effect of training ($p \leq 0.05$) irrespective of group. † indicates a significant interaction effect ($p = 0.031$).

Table 6.3. Army physical fitness test parameters across training weeks organised by group. Values are means \pm SD.

Physical Fitness Variable	Smoking Status (n)	Week of training	
		1	14
Press ups*	NS (20)	49.5 \pm 14.8	62.0 \pm 13.6
	S (21)	47.0 \pm 16.0	61.3 \pm 9.2
Sit ups*	NS (20)	61.8 \pm 16.1	70.1 \pm 11.6
	S (21)	55.0 \pm 9.3	67.9 \pm 8.5
Run time (min:sec)* †	NS (19)	10:08 \pm 00:59	9:26 \pm 00:38
	S (21)	10:07 \pm 00:46	9:48 \pm 00:32

Note: *denotes a significant effect of training ($p \leq 0.05$) irrespective of group. † signifies a non-significant interaction trend ($p = 0.067$)

Table 6.4. pQCT data of the lower leg across training weeks organised by group (NS, n=22; S, n=23). Data are means \pm SD.

Variable	Smoking Status	Week of training	
		1	10
Muscle Area (mm ²)*	NS	8110 \pm 895	8510 \pm 1023
	S	8354 \pm 766	8698 \pm 779
Fat Area (mm ²)	NS	1753 \pm 631	1646 \pm 496
	S	1857 \pm 584	1791 \pm 576
Fat/Muscle Area Ratio (%)*	NS	22.0 \pm 8.8	19.9 \pm 7.4
	S	22.3 \pm 7.0	20.7 \pm 7.0
Muscle Density (mg.cm ⁻³)	NS	75.7 \pm 2.2	75.9 \pm 1.5
	S	75.9 \pm 1.0	76.1 \pm 1.6
Total Density (mg.cm ⁻³)*	NS	66.5 \pm 4.6	67.2 \pm 3.6
	S	65.8 \pm 4.3	66.7 \pm 3.8

Note: * denotes a significant effect of training ($p \leq 0.05$) irrespective of group

Table 6.5. Lung function parameters across training weeks organised by group. Values are means \pm SD.

Lung Function Variable	Smoking Status (n)	Week of training	
		1	10
FEV1 (L)	NS (21)	4.2 \pm 0.6	4.1 \pm 0.7
	S (24)	4.2 \pm 0.4	4.2 \pm 0.4
FVC (L)*	NS (21)	4.7 \pm 0.9	4.5 \pm 1.0
	S (24)	5.1 \pm 0.5	4.7 \pm 0.5
FER (%)*#	NS (21)	88.5 \pm 7.3	92.2 \pm 8.4
	S (24)	83.5 \pm 5.0	88.8 \pm 6.6
PEF (mL ⁻¹)*	NS (20)	529 \pm 104	560 \pm 105
	S (24)	487 \pm 116	528 \pm 80

Note: * denotes a significant effect of training ($p \leq 0.05$) irrespective of group. # signifies a significant effect of smoking status ($p \leq 0.05$)

6.3.10 Endocrine markers

Serum testosterone concentrations for weeks 1, 5 and 10 for non-smokers were 10.62 (± 0.68), 10.53 (± 0.58) and 11.40 (± 0.59) ng·mL⁻¹, respectively and 11.27 (± 0.57), 10.39 (± 0.62) and 11.10 (± 0.62) ng·mL⁻¹, respectively, for smokers. No statistically significant effects were found ($p > 0.05$). Testosterone concentrations for weeks 1, 5 and 10 for subgroups were not significantly different irrespective of time, and contained no significant interaction effect ($p > 0.05$). Subgroup analysis did reveal a significant effect of training ($p = 0.03$) whereby irrespective of group, week 5 values were lower than those of weeks 1 and 10 (Figure 6.6; Panel A).

Cortisol concentrations for weeks 1, 5 and 10 for non-smokers were 145 (± 7), 144 (± 4) and 131 (± 6) ng·mL⁻¹, respectively and 143 (± 7), 149 (± 6) and 136 (± 8) ng·mL⁻¹, respectively, for smokers. Cortisol concentrations for subgroups are presented in Figure 6.6 (Panel B). Both comparisons contained a significant training effect from reduction between week 5 and week 10 ($p < 0.05$), but no significant interaction or group effects were present ($p > 0.05$).

Serum IGF-1 concentrations for weeks 1, 5 and 10 for non-smokers were 270 (± 14), 249 (± 10) and 264 (± 14) ng·mL⁻¹, respectively and 267 (± 12), 242 (± 11) and 242 (± 10) ng·mL⁻¹, respectively, for smokers. Serum IGF-1 concentrations for non-smokers, light, moderate and heavy smokers are presented in Figure 6.7. In both analyses, irrespective of group, IGF-1 significantly decreased from baseline to week 5 ($p < 0.01$). No significant interaction or group effects were found ($p > 0.05$).

5.3.11 Intramuscular proteins and enzymes

Mean values (\pm SE) for myoglobin in weeks 1, 5 and 10 were 57.3 (± 5.8), 89.3 (± 12.4) and 73.6 (± 8.2) ng·mL⁻¹ for non-smokers ($n=22$), and 49.6 (± 3.8), 69.7 (± 4.7) and 55.0 (± 1.9) ng·mL⁻¹ for smokers ($n=23$), respectively. No significant interaction or group effects existed ($p > 0.05$), but a significant effect of training was present ($p < 0.001$). Post hoc analyses showed significant differences between all time points irrespective of group. For both groups, myoglobin peaked in week 5 and then decreased in week 10 to values between those measured in weeks 1 and 5. In non-smokers, baseline values were similar to those of week 5 in smokers.

Mean values (\pm SE) for creatine kinase in weeks 1, 5 and 10 were 200 ± 29 , 407 ± 45 and 350 ± 47 U·L⁻¹ for non-smokers ($n=21$), and 252 ± 69 , 395 ± 38 and 232 ± 22 U·L⁻¹ for smokers ($n=24$), respectively. Creatine kinase followed a similar pattern to myoglobin across time points; peaking in week 5. Statistical analysis showed a significant effect of training ($p < 0.001$) irrespective of group. Post hoc analyses demonstrated the training effect comprised significant differences between weeks 1 and 5, and between weeks 5 and 10. No other significant interaction or group effects were present.

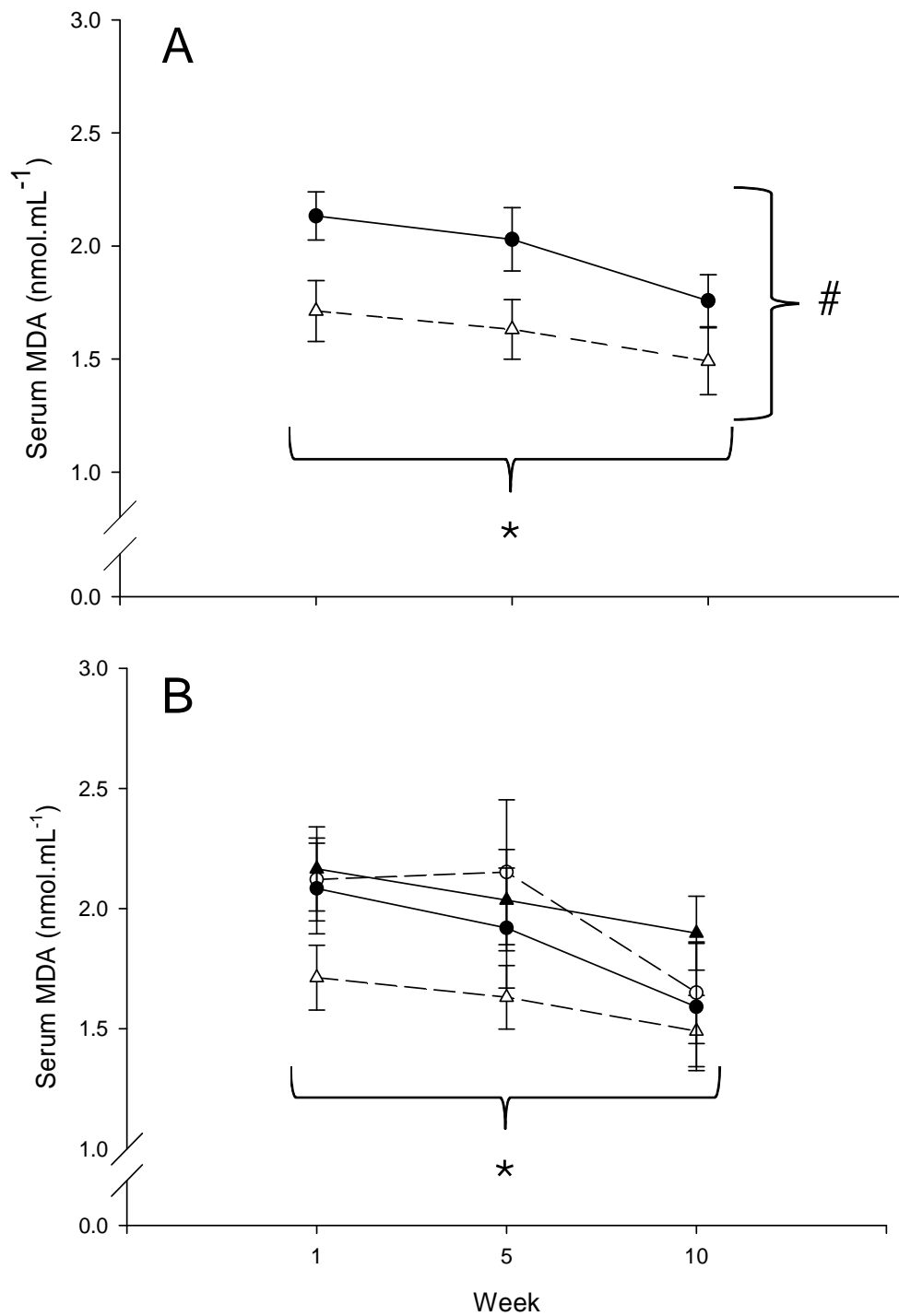


Fig 6.1. Mean serum concentration of MDA (nmol·mL⁻¹) across weeks 1, 5 and 10 of training. A: Groups NS (Open triangle) and S (Closed circle). B: Groups NS (Open triangle), LS (Open circle), MS (Closed triangle) and HS (Closed circle). Values are means with standard error bars. #and parentheses denote significant effect of smoking status ($p=0.026$). *and parentheses denote a significant effect of training ($p<0.05$).

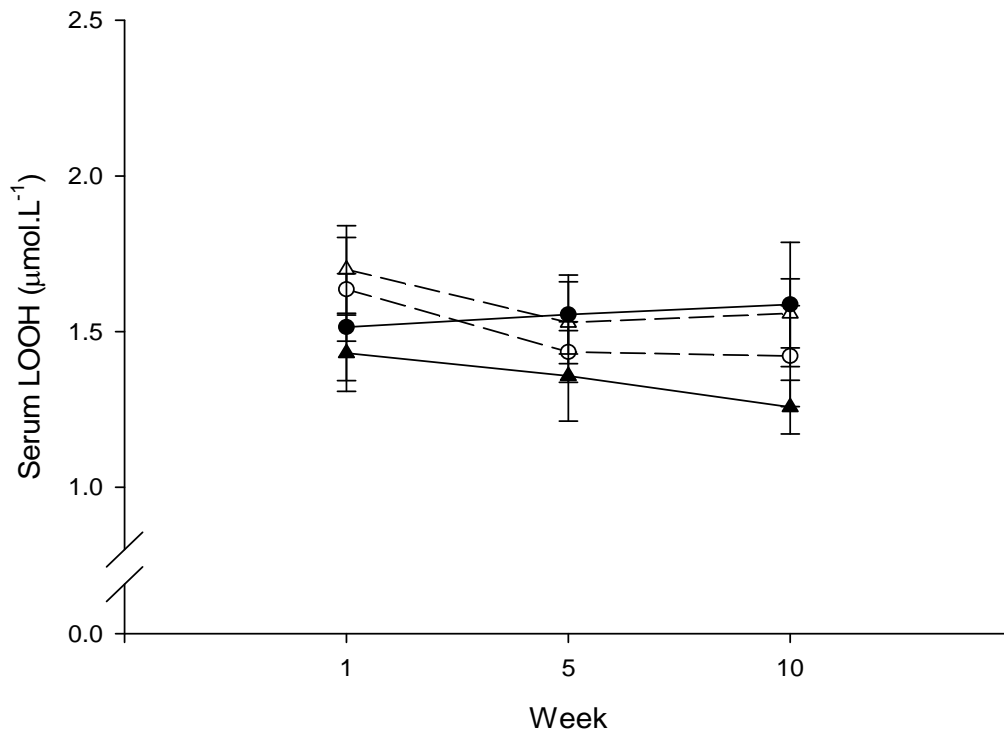


Fig 6.2. Mean serum concentration of LOOL ($\mu\text{mol}\cdot\text{L}^{-1}$) in groups NS (Open triangle), LS (Open circle), MS (Closed triangle) and HS (Closed circle) across weeks 1, 5 and 10 of training. Values are means with standard error bars.

6.3.12 Full blood count

The population of neutrophils was shown to be significantly greater in smokers irrespective of training ($p=0.035$). ANOVA showed that a significant training effect ($p<0.05$) was present in all haematological factors over training weeks with the exception of populations of neutrophils and eosinophils. Full blood count results are summarised in Table 6.6.

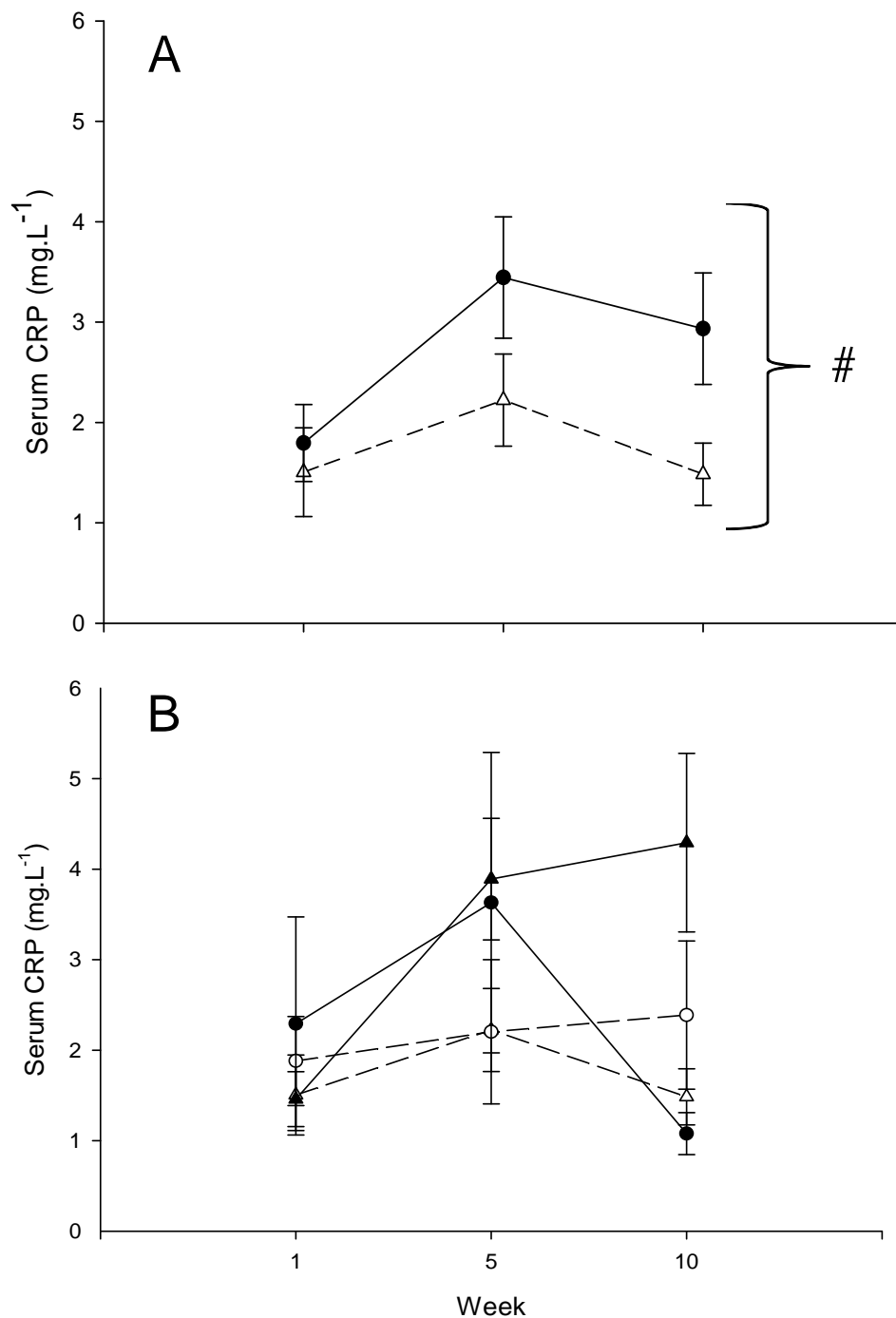


Fig 6.3. Mean serum concentration of CRP ($\text{mg}\cdot\text{L}^{-1}$) across weeks 1, 5 and 10 of training. A: Groups NS (Open triangle) and S (Closed circle). B: Groups NS (Open triangle), LS (Open circle), MS (Closed triangle) and HS (Closed circle). Values are means with standard error bars. # and parentheses denote significant effect of smoking status ($p=0.047$).

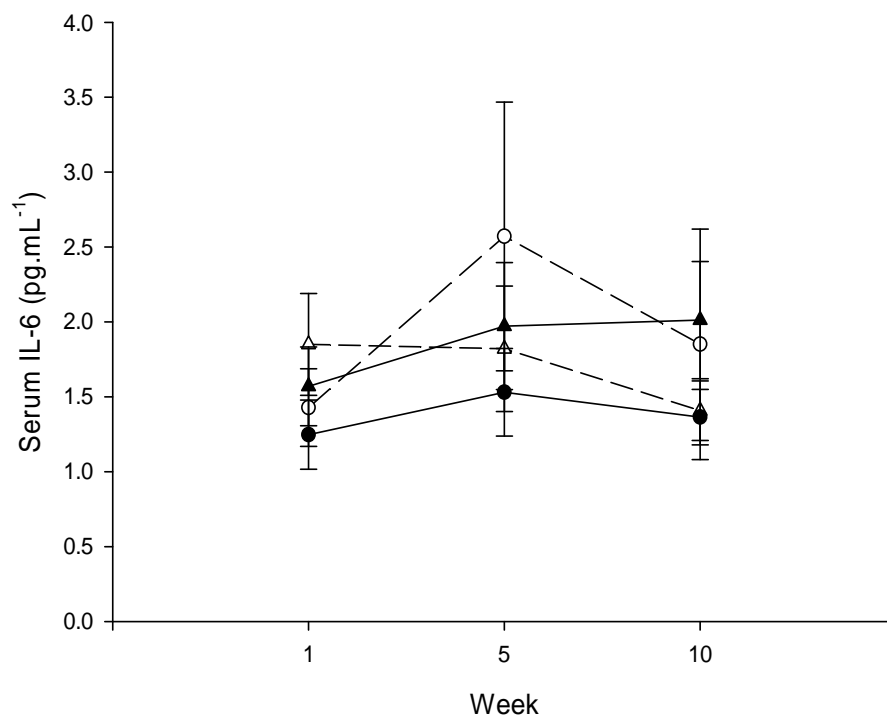


Fig 6.4. Mean serum concentration of IL-6 ($\text{pg}\cdot\text{mL}^{-1}$) between groups NS (Open triangle), LS (Open circle), MS (Closed triangle) and HS (Closed circle) across weeks 1, 5 and 10 of training. Values are means with standard error bars.

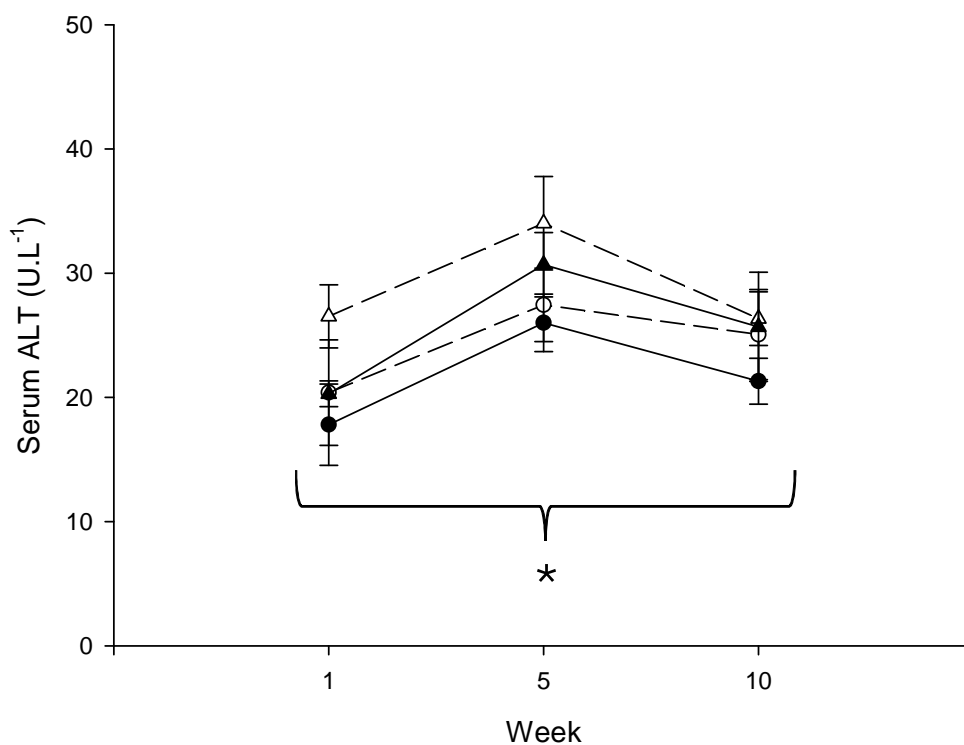


Fig 6.5. Mean serum ALT activity ($\text{U}\cdot\text{L}^{-1}$) between groups NS (Open triangle), LS (Open circle), MS (Closed triangle) and HS (Closed circle) across weeks 1, 5 and 10 of training. Values are means with standard error bars. *and parentheses denote a significant effect of training ($p<0.001$).

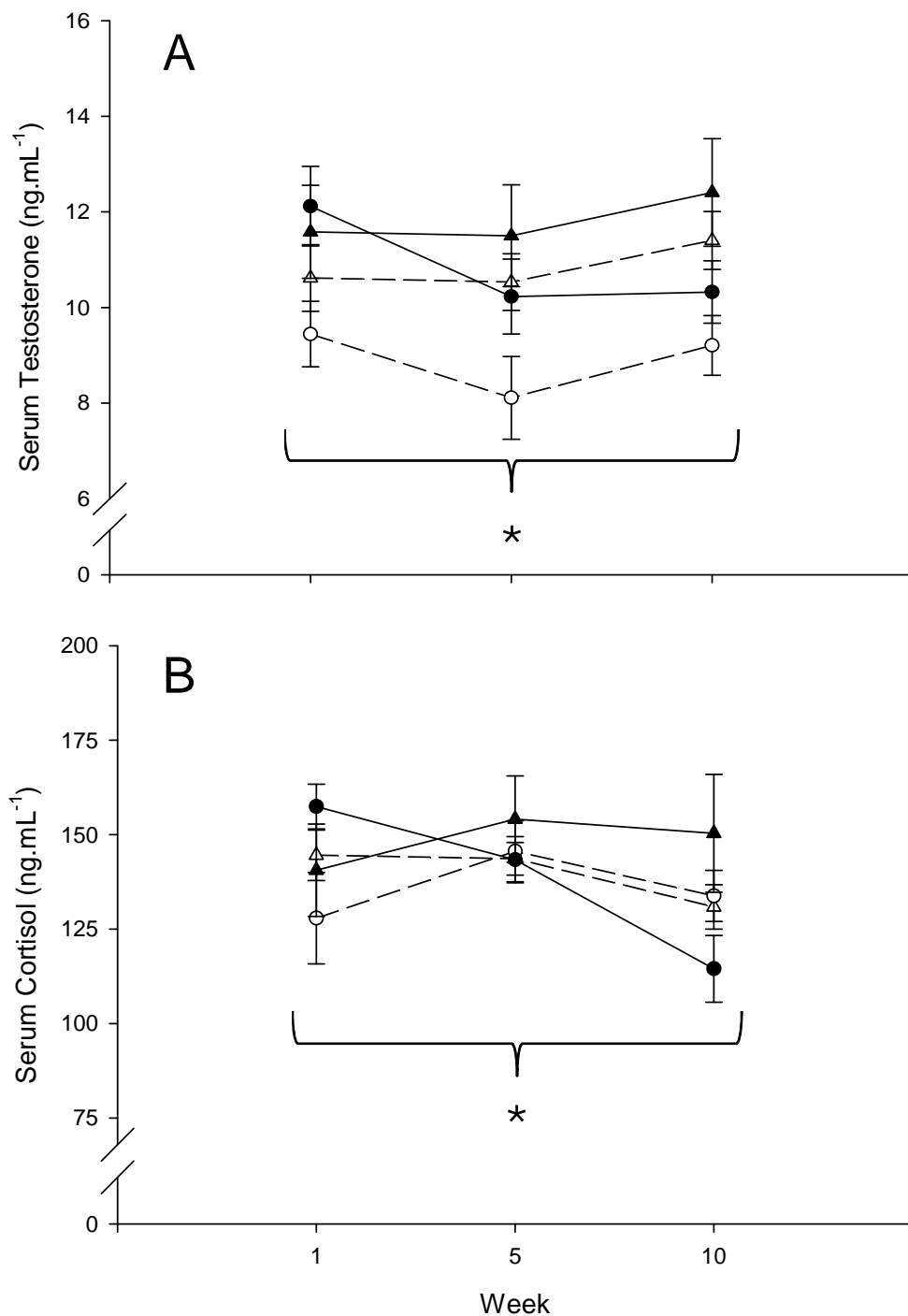


Fig 6.6. Comparison of mean serum concentration of testosterone (A; $\text{ng}\cdot\text{mL}^{-1}$) and cortisol (B; $\text{ng}\cdot\text{mL}^{-1}$; NS, $n=23$; S, $n=40$) determined by immunoassay between groups NS (Open triangle), LS (Open circle), MS (Closed triangle) and HS (Closed circle) across weeks 1, 5 and 10 of training. Data are presented as means with standard error bars. *and parentheses denote a significant effect of training ($p < 0.05$).

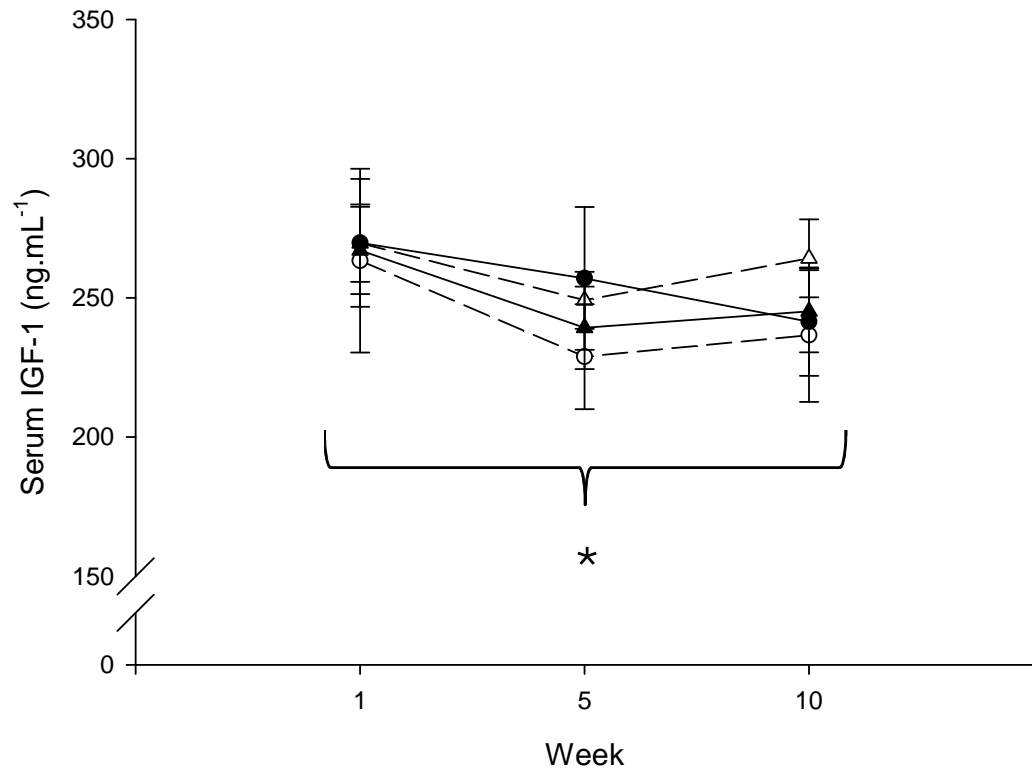


Fig 6.7. Mean serum concentration of IGF-1 ($\text{ng}\cdot\text{mL}^{-1}$) between groups NS (Open triangle), LS (Open circle), MS (Closed triangle) and HS (Closed circle) across weeks 1, 5 and 10 of training. *and parentheses denote a significant effect of training ($p < 0.001$)

Table 6.6. Full blood count data. Populations of cell types across training weeks organised by smoking status (NS, n=17; S, n=20). Data are means \pm SE

Variable	Smoking Status	Normal Range	Week of training		
			1	5	10
(HB) Haemoglobin (g.dL ⁻¹)*	NS	13-18	15.5 \pm 0.3	14.5 \pm 1.0	14.8 \pm 0.2
	S		15.1 \pm 0.2	14.6 \pm 0.1	14.5 \pm 0.2
WBC (10 ⁹ ·L ⁻¹)	NS	4-11.0	7.92 \pm 0.47	7.33 \pm 0.47	7.39 \pm 0.38
	S		8.42 \pm 0.33	8.96 \pm 0.67	8.18 \pm 0.42
(PLT) Platelets (10 ⁹ ·L ⁻¹)*	NS	150-400	247 \pm 13	280 \pm 12	270 \pm 10
	S		263 \pm 13	324 \pm 24	297 \pm 14
RBC (10 ¹² ·L ⁻¹)*	NS	4.5-6.5	5.2 \pm 0.1	4.9 \pm 0.1	5.0 \pm 0.1
	S		5.0 \pm 0.1	4.9 \pm 0.1	4.9 \pm 0.1
(HCT) haematocrit (%)*	NS	0.40-0.54	0.43 \pm 0.01	0.41 \pm 0.01	0.43 \pm 0.01
	S		0.43 \pm 0.00	0.42 \pm 0.00	0.42 \pm 0.00
(MCV) Mean corpuscular volume (fl)*	NS	78-98	82.9 \pm 0.6	84.1 \pm 0.8	85.0 \pm 0.9
	S		85.5 \pm 0.8	86.0 \pm 0.8	85.9 \pm 0.8
(MCH) Mean corpuscular haemoglobin (pg)*	NS	27.5-32.5	29.9 \pm 0.3	29.6 \pm 0.3	29.4 \pm 0.3
	S		30.4 \pm 0.3	30.1 \pm 0.3	29.6 \pm 0.3
Neutrophils (10 ⁹ ·L ⁻¹)#	NS	2-7.5	3.86 \pm 0.37	3.85 \pm 0.38	3.63 \pm 0.26
	S		4.44 \pm 0.26	5.31 \pm 0.60	4.57 \pm 0.38
Lymphocytes (10 ⁹ ·L ⁻¹)*	NS	1.5-4.0	3.01 \pm 0.28	2.48 \pm 0.22	2.85 \pm 0.26
	S		2.85 \pm 0.16	2.49 \pm 0.14	2.60 \pm 0.16
Monocytes (10 ⁹ ·L ⁻¹)*	NS	0.2-0.8	0.61 \pm 0.04	0.61 \pm 0.04	0.52 \pm 0.03
	S		0.70 \pm 0.05	0.76 \pm 0.06	0.62 \pm 0.05
Eosinophils (10 ⁹ ·L ⁻¹)	NS	0.0-0.4	0.24 \pm 0.03	0.21 \pm 0.02	0.09 \pm 0.02
	S		0.26 \pm 0.03	0.24 \pm 0.02	0.26 \pm 0.02
Basophils (%)*	NS	0.0-0.2	0.65 \pm 0.16	0.15 \pm 0.11	0.28 \pm 0.15
	S		0.41 \pm 0.13	0.26 \pm 0.10	0.23 \pm 0.10

Note: * denotes a significant effect of training ($p \leq 0.05$) # signifies a significant effect of smoking status ($p \leq 0.05$).

6.4 Discussion

The current study was designed to examine differences in physical performance, muscular adaptation, oxidative stress, inflammatory markers and hormones between smokers and non-smokers in response to 10 weeks of military training. Further to this, smokers were separated into subgroups classified by cigarette consumption to examine whether a dose-response existed in these biochemical markers during training. The main findings of the study were that performance improvement and muscular adaptation were evident as a result of military training irrespective of group, but adaptations in most physical performance parameters were not different between smokers and non-smokers. There was, however, a non-significant trend for improvement in run time to be greater in non-smokers. Concentrations of the oxidative stress marker MDA and the acute phase protein CRP were significantly higher in smokers irrespective of time. Smoking status imparted no significant effect on resting concentrations of the other measured endocrine or inflammatory parameters and the existence of a dose-response to smoking in biochemical markers was not supported.

Compared to non-smokers, habitual smokers are reported to have reduced physical fitness (Hirsch et al. 1985; Bernaards et al. 2003; Kobayashi et al. 2004) and impaired run performance in military training (Marti et al. 1988; Haddock et al. 2007). However, only one study has examined changes in physical performance in response to a standardised training programme in smokers and non-smokers, demonstrating significantly impaired adaptation in smokers over 6 months of Army officer cadet training (Hoad & Clay 1992). In contrast, the current study observed little effect of habitual smoking on improvement in performance variables. A trend for non-smokers to have greater improvement in run performance was observed but was not significant ($p=0.067$). In comparison to the above study, the duration of the current study is shorter, which alongside differences in training environment and training itself, might explain why similar findings were not observed. In light of this, however, it is possible that if the trend for impaired improvement in run performance continued over the entire 26 week training course, the difference between habitual smokers and non-smokers might increase. Bench press performance showed greater improvement in smokers than non-smokers, but this

could be a product of some individuals partaking in recreational weight training, given the absence of upper body maximal strength training in the military course.

As expected, military training elicited muscular adaptation in the lower leg. The observed increase in muscle area and decrease in fat-to-muscle ratio were concurrent with whole body anthropometric data, which indicated an overall increase in lean mass. These findings demonstrate changes expected as a result of endurance training (Williams 2005). Additionally, a novel finding of the study was that height decreased during training, which could be a function of extended periods involving heavy load carriage. The study rationale proposed that habitual smoking may impair muscle cell signalling and recovery from exercise, potentially causing less pronounced muscular hypertrophy from training in smokers. Muscle characteristics, unlike performance variables, could not be confounded by fatigue or lethargy and given adaptations in smokers and non-smokers were similar, provide little evidence for an influence of smoking on muscle adaptation to training.

Malondialdehyde (MDA), a marker of oxidative stress, was significantly higher in smokers than non-smokers, irrespective of training. As MDA is a relatively stable end-product of lipid peroxidation, its elevation is considered to be associated with long-term oxidative stress (Del Rio et al. 2005). Typically, circulatory markers of oxidative stress are chronically elevated in habitual smokers in comparison to non-smokers (Reilly et al. 1996; Helmersson et al. 2005; Tanriverdi et al. 2006; Isik et al. 2007), in agreement with the present study and the previous chapter. Some authors have suggested that in muscle an upper limit for oxidative stress exists (Andrade et al. 2001), above which the beneficial effects of redox signalling on muscular contractility and adaptation to exercise are disrupted (Andrade et al. 1998; Andrade et al. 2001; Close et al. 2005; Yamada, Mishima, et al. 2006). If a circulatory elevation in ROS influences local accumulation of oxidants then the pro-adaptive process to exercise training may be disrupted in smokers. The current study suggests, however, that chronically elevated circulatory ROS did not impart this effect, as performance improvements and muscular adaptations were similar in smokers and non-smokers. Conversely, lipid hydroperoxides (LOOH), typically associated with short-term increases in oxidative stress, were similar in smokers and non-smokers. However, as acute markers of oxidative stress rise in response to smoking an individual cigarette (Morrow et al.

1995), this finding may be explained by overnight cessation from smoking in the present study, prior to collecting blood samples.

An elevation of MDA alone over time suggests that habitual smoking increases lipid peroxidation via oxidative stress, with potential implications for increased systemic inflammation (Van der Vaart et al. 2004). Equally, it has also been postulated that a chronic state of low-grade inflammation, like that reported previously in smokers (Bazzano et al. 2003; O'Loughlin et al. 2008), may serve to increase oxidative stress by increased phagocytic production of ROS as part of host defence (Cross et al. 1998). In our previous study, no significant differences were found in CRP between smokers and non-smokers at entry to training, despite the existence of elevated resting MDA. In the current study, CRP levels in both groups were also similar at baseline but became distinct at weeks 5 and 10, suggesting that military training evokes an increase in resting inflammation, and that this is greater in habitual smokers. At weeks 5 and 10 smokers exhibited concentrations of CRP greater than or close to $3 \text{ mg}\cdot\text{L}^{-1}$, which is higher than is expected for a group of this age and activity level (Woloshin & Schwartz 2005). These results suggest that military training elicited an inflammatory response which was exacerbated or persisted above normal due to the presence of elevated oxidative stress in smokers.

Elevated CRP in the circulation of smokers in the absence of elevated IL-6 could be explained by two hypotheses. First, in a chronic inflammatory state, the two markers can be elevated independently of one another (Dixon et al. 2009), though underlying mechanisms have not been elucidated. Oxidative stress, and associated oxidative damage, could potentially provide a stimulus for CRP remaining in circulation without the stimulation of IL-6 (Van der Vaart et al. 2004; Helmersson et al. 2005; Yanbaeva et al. 2007). However, MDA decreased between weeks 1 and 10, which is a different time-course from CRP, possibly indicating the changes in these markers are not responsible for one another. Second, observed CRP concentrations could indicate acute inflammatory responses to training stimuli. As CRP possesses a longer half-life than IL-6, it could be present in circulation longer after initial stimulation from IL-6 (Heinrich et al. 1990). This notion would also correspond with similar CRP levels in smokers and non-smokers at baseline, before training commenced. Factors associated with military training environments may incite acute inflammatory responses, such as reduced sleep duration (Booth et

al. 2006), high incidence of minor infections (Blacker et al. 2005) and intense or unaccustomed exercise training. In either case, the findings suggest that there is a greater tendency for inflammation in habitual smokers than non-smokers in response to the factors described above, or that smoking itself incites substantial daily inflammatory responses when combined with training.

Although long term exercise training results in antioxidant-like processes (Gomez-Cabrera, Domenech & Viña 2008) and a decrease in CRP levels over time (Plaisance & Grandjean 2006; Wilund 2007), it is unknown whether elevated CRP and MDA indicate a state that would be detrimental to physical fitness development. Given current findings, the elevated levels of MDA and CRP in smokers over 10 weeks of training could only have potentially influenced endurance exercise. As discussed earlier, oxidative stress can have local inhibitory effects on muscle function (Andrade et al. 1998; Moopanar & Allen 2005), potentially accelerating muscular fatigue (Morse et al. 2007; Wüst et al. 2008). This may be particularly relevant when comparatively greater increases in oxidative stress in response to graded treadmill running have been observed in young smokers (Bloomer et al. 2007). Oxidative stress in smokers may affect performance in endurance exercise that cumulatively influences long term improvement in performance. A distinct lack of research into the effect of chronically elevated CRP on muscular or cardiorespiratory development means it is unclear how it may have influenced the adaptive response to military training.

The days prior to blood sampling in week 5 contained more arduous training than those for week 1 or week 10, which was reflected in significant decreases in testosterone and IGF-1, increases in ALT and markers potentially indicating muscle damage, and peaks in CRP and cortisol. The changes in these markers are similar to those observed in frequent periods of energy deficit, intense physical activity and low sleep quality during military training (Nindl et al. 2003; Booth et al. 2006; Nindl, Barnes, et al. 2007; Kyröläinen et al. 2008; Tyyskä et al. 2010). Previously, both short and long term military training have resulted in hormonal changes tending towards an ostensibly catabolic state, presenting decreased concentration and bioavailability of IGF-1, while increasing circulating cortisol (Nindl et al. 2003; Tanskanen, Uusitalo, et al. 2011). Currently, the exact role of hormones in regulation of muscle mass and long-term exercise adaptation are participant to great

debate (Urhausen, Gabriel & Kindermann 1995; Izquierdo et al. 2006; Spangenburg et al. 2008; West et al. 2009). However, changes in resting hormone concentrations are likely to be reflective of recent periods of intense training and therefore might be useful in future as indicators of the effectiveness of adaptation following that period of training.

Hormone concentrations were not different in smokers *versus* non-smokers, or as a result of greater smoking exposure. Previous research in habitual smokers consistently demonstrates increased blood or salivary cortisol concentrations (Kirschbaum et al. 1992; Field et al. 1994; Steptoe & Ussher 2006) and decreased endogenous IGF-1 (Holmes et al. 2002; Renehan et al. 2004), while the effects on testosterone have presented mixed results (Zmuda et al. 1997; English et al. 2001; Svartberg & Jorde 2007; Richthoff et al. 2008). However, no studies have measured the effects of training on resting hormone concentrations within smokers and non-smokers. It is possible that smoking does not affect waking concentrations of hormones, but overall secretion, secretion at specific times of the day, or acutely following exercise. As such, the collection of blood samples only at waking may have limited the observation of any influence of smoking on hormones that follow a circadian rhythm. Additionally, the effect of smoking on IGF-1 and testosterone may be manifest within the production of transport proteins and subsequent bioavailability as opposed to total concentration (Kaklamani et al. 1999; Steptoe & Ussher 2006).

An extended period of exercise training would ordinarily improve lung function variables. As a result of training, forced expiratory ratio and peak expiratory flow increased significantly, irrespective of group. Forced vital capacity, however, decreased. Additionally, the adverse effect of smoking is typically evident on all lung function variables, but only forced expiratory ratio was affected, indicating that smoking did not substantially influence lung function. Neutrophil population was significantly increased in smokers over training duration ($p < 0.05$), in agreement with the purported up-regulation of these cells first in lung tissue and subsequently in the circulation in response to smoking (Taylor, 2010). This further supports that smoking did not have as substantial effect as expected on immune response and lung health, potentially due to the youth and activity level of the population.

The present study contained several factors that may have influenced the effect of smoking on development of physical fitness and on examined biochemical markers. It is apparent that 10 weeks of training may not be long enough to identify differing responses to exercise training between smoking groups. Additionally, it may be that positive changes in physical fitness imparted by military training are substantial enough to mask what small adverse effects smoking may have on physical fitness development. Similarly, it should be noted that the original sample size in this study was 107 and reduced to a sample of 46 owing to drop out from military training from injury, unacceptable physical performance, military discharge or participant choice. As such, it may be that the sample is unintentionally biased towards those who have adapted more positively to training.

Physical performance improvement was evident irrespective of group across training weeks and was indicative of military-type tasks such as load-bearing marches. No effects of habitual smoking on muscular adaptation parameters or physical performance measures were apparent with the exception of a non-significant trend for greater run time improvement in non-smokers. Elevated oxidative stress in smokers may have exacerbated inflammatory responses to military training, demonstrated by elevated CRP. However, it is impossible to establish causality, and it is likely that the complex interplay of inflammation and oxidative stress during training cannot be fully understood from the current findings. Aside from MDA and CRP, other inflammatory markers and hormones which were expected to be altered in smokers were found to be similar to non-smokers. Given that overall fitness did not differ between smokers and non-smokers, this may indicate that the relative youth and limited years of smoking exposure of participants could explain smokers not exhibiting different performance adaptation and endocrine and inflammatory markers from non-smokers. Habitual smoking appears to have a profound effect on chronically elevating oxidative stress and, during training, exacerbating inflammation, but neither process appears to influence muscular adaptation or improvement in physical fitness.

CHAPTER 7

Study 4

The Influence of Smoking on Military Physical Fitness Test Performance during Initial Training

7.1 Introduction

Smoking prevalence in military populations is typically greater than that of the general population (Chapter 4; Fear et al. 2010). Epidemiological evidence exists indicating habitual smoking is associated with lower physical fitness (Bernaards et al. 2003; Kobayashi et al. 2004) and performance in strength tasks (Al-Obaidi et al. 2004). In a military setting, regular smoking has been reported to be predictive of lower physical fitness at entry to training (Haddock et al. 2007), to adversely affect athletic performance during training (Zadoo et al. 1993) and to result in poorer training outcomes (Marti et al. 1988). Lower physical fitness in habitual smokers at entry to training could be explained, in part, by smokers also typically having lower physical activity and participation in exercise compared to non-smokers (Larsson & Orlander 1984; Larson et al. 2007). Once in prolonged standardised training, however, little evidence exists as to whether habitual smoking directly affects the development of physical fitness.

Improvement in physical performance over a six month officer training programme was significantly greater in non-smokers when compared to habitual smokers (Hoad & Clay 1992). Contrary to this, similar research studying British infantry trainees found no significant differences between smokers and non-smokers in muscular adaptation or improvement in physical performance tests during 14 weeks of training (Chapter 6). It was noted, however, that trends existed for smaller improvement in run performance in smokers that may lead to poorer training outcomes over a greater training duration. To date, no further research has been completed to further test this hypothesis.

Military training, with long-duration standardised training programmes, regular physical fitness testing and high levels of physical activity, is a suitable platform for testing development of physical fitness in a large sample. Additionally, the relatively high prevalence of smoking in this population gives an opportunity to study the effect of smoking on long term training. As such, this

study aims to explore whether habitual smoking impairs improvement in performance of military physical fitness tests during 24 weeks of initial training in a large sample of British infantry trainees.

7.2 Methods

7.2.1 Participants

A cohort of male British infantry trainees (n=2087) took part in this study.

7.2.2 Study design

Performance in routine military fitness tests at weeks 1, 14 and 24 were collected for trainees who completed a military pre-training questionnaire to determine smoking status and self-reported physical activity level prior to entry to training. Two analyses were completed using 1) all trainees observed and 2) all trainees who completed training with physical performance data at each time point.

7.2.3 Physical performance test data

Military fitness tests consisted of press ups and sit ups when allowing 2 minutes for each exercise, and a best effort 2.4 km run.

As expected over the 24 week course, substantial drop out occurred causing each time point to include fewer participants. Additionally, owing to circumstances such as injury or illness some participants did not complete every test within each stage of physical performance testing.

7.2.4 Statistical analysis

Statistical analyses were completed using PASW 18.0 for Windows (SPSS Inc, Chicago, Illinois). A linear mixed model (LMM) was used to identify any significant differences between non-smokers and smokers in the full data set and significant effects of time or interaction. LMM has been shown to be an appropriate statistical test to account for missing data. A first order auto-regressive structure (AR(1)) was chosen to model variance, which assumes that values will be less correlated with one another if further apart in time. This structure produced the lowest Akaike Information Criterion, demonstrating the most appropriate goodness of fit for the data. Physical activity score for each participant was entered in the linear mixed model as a covariate. The LMM used all observations in all time points to model the relationship over time and produce estimated marginal means for each variable for weeks 1, 14 and 24. A two-way mixed model ANOVA was used to identify significant group, time or interaction effects when only including those who reached the end of training. Post-hoc analysis with stepwise bonferroni adjustment was used to determine the location of variance in the event of a significant interaction or training effect when analysing more than two time points or groups. Greenhouse Geisser ANOVA outputs were used in cases where Mauchley's test of sphericity showed data to be aspherical. Statistical significance was identified at $p < 0.05$. Data are presented as estimated marginal mean \pm SE (with self-reported physical activity score as a covariate) for results of LMM and mean \pm SE for results analysed by ANOVA.

7.3 Results

From the original sample of 2087 trainees physical fitness test data could be obtained for 1182 (707 smokers) trainees in week 1, 896 (529 smokers) trainees in week 14 and 755 (421 smokers) trainees in week 24. Exact sample numbers used in the linear mixed model are presented in Table 7.1, grouped by performance variables.

7.3.1 Military physical fitness test performance

Estimated marginal means (\pm SE) for number of press ups performed for weeks 1, 14 and 24 for non-smokers (48.3 (\pm 0.6), 54.6 (\pm 0.7), 57.0 (\pm 0.7)) and smokers (44.2 (\pm 0.5), 51.8 (\pm 0.5), 54.5 (\pm 0.6)), are shown in Figure 7.1 (Panel A). Figure 7.2 (Panel A) displays the estimated marginal means for sit up performance for weeks 1, 14 and 24 for non-smokers (57.5 (\pm 0.5), 62.8 (\pm 0.6), 66.0 (\pm 0.6)) and smokers (53.9 (\pm 0.4), 60.6 (\pm 0.5), 63.2 (\pm 0.5)). Estimated marginal means for 2.4 km run performance for weeks 1, 14 and 24 in non-smokers (612 (\pm 2), 579 (\pm 2) and 567 (\pm 2) secs) and smokers (622 (\pm 2), 586 (\pm 2) and 571 (\pm 2) secs) are displayed in Figure 7.3 (Panel A). LMM analysis demonstrated significant group effects in all physical performance measures, such that non-smokers performed better at all time points ($p < 0.01$). Additionally, a significant effect for improvement in performance over time for all physical performance variables existed, irrespective of group ($p < 0.01$). No interaction effects were present ($p > 0.05$).

When only including individuals with complete data sets, ANOVA identified significant time and group effects in press ups (Figure 7.1; Panel B), sit ups (Figure 7.2; Panel B) and run performance (Figure 7.3; Panel B) ($p < 0.05$). ANOVA also identified a significant interaction effect in run performance in those who finished training but post-hoc adjustment meant the individual data points were not significantly different ($p > 0.05$).

Table 7.1. Participant numbers organised by smoking status and total number of observations for PFT results.

Variable	Week 1		Week 14		Week 24		Total Observations
	NS	S	NS	S	NS	S	
Press up	475	707	367	528	301	423	2801
Sit up	475	707	367	529	302	424	2804
Run	472	701	334	493	334	421	2755

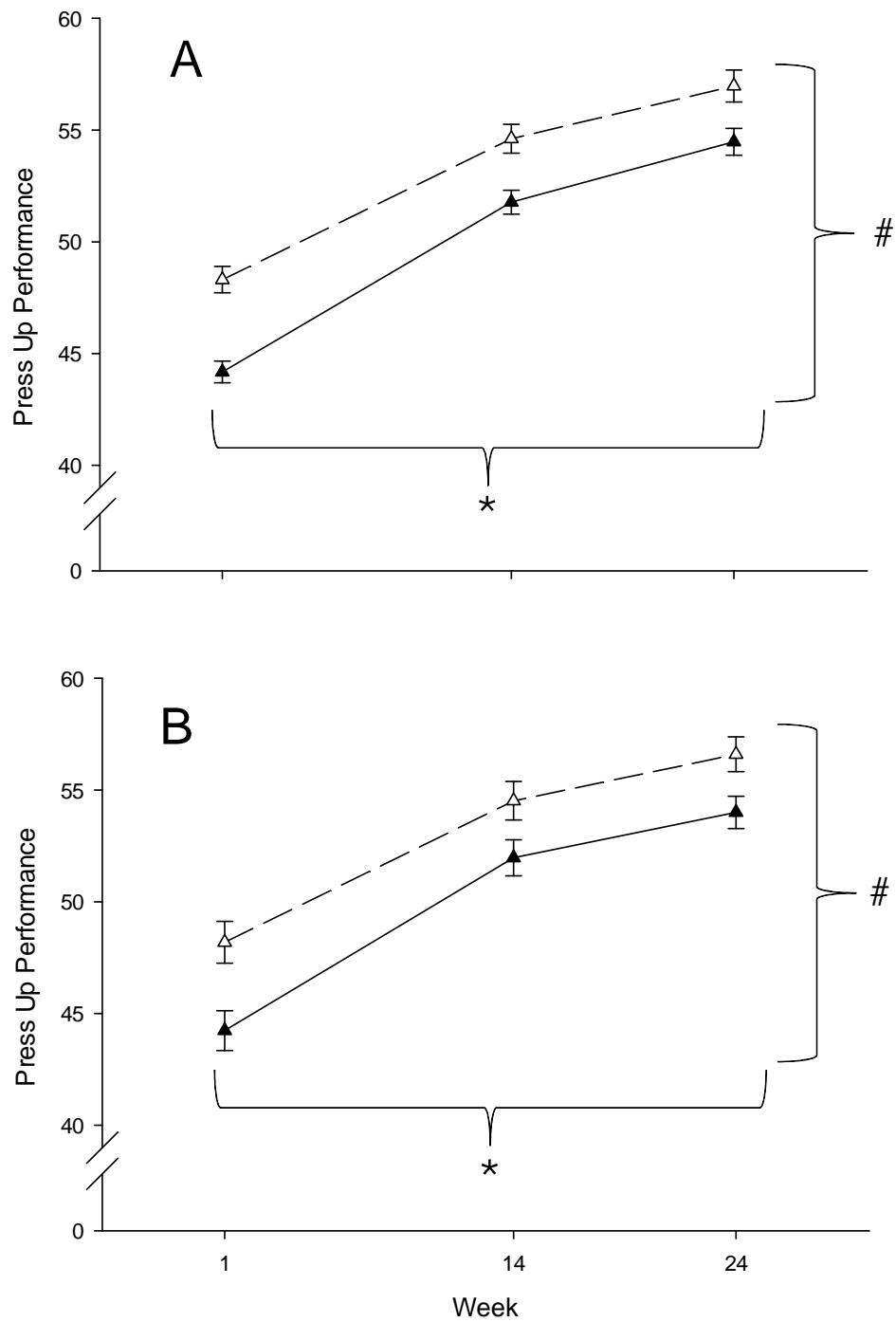


Fig 7.1. Press ups completed in two minutes in groups NS (Open triangle) and S (Closed triangle) at weeks 1, 14 and 24 of training. A: Values are estimated marginal means with standard error bars for all participants. B: Values are means with standard error bars for all participants with complete data sets. *significant effect of time, irrespective of group ($p < 0.01$). #significant effect of smoking status, irrespective of time ($p < 0.01$).

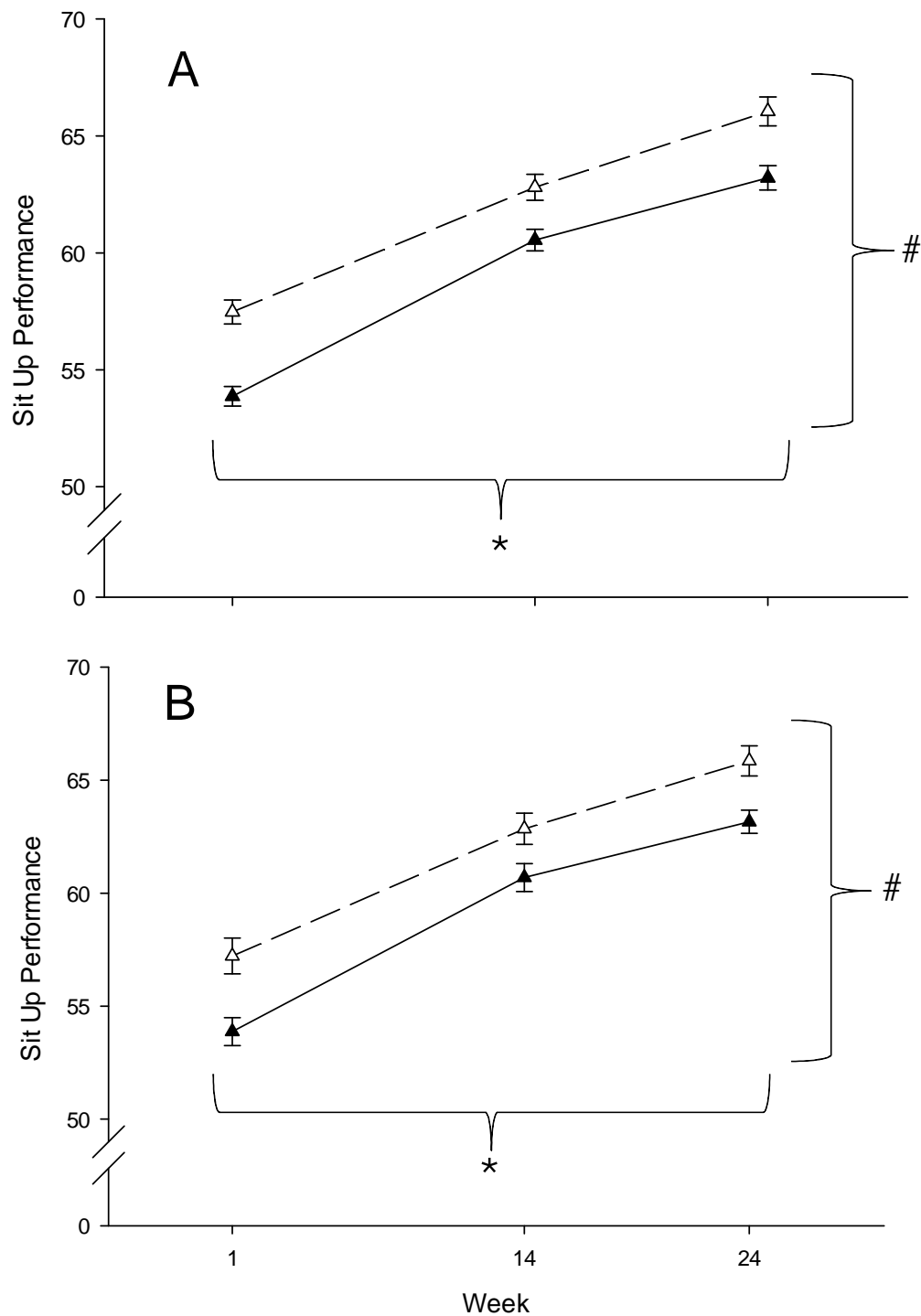


Fig 7.2. Sit ups completed in two minutes in groups NS (Open triangle) and S (Closed triangle) at weeks 1, 14 and 24 of training. A: Values are estimated marginal means with standard error bars for all participants. B: Values are means with standard error bars for all participants with complete data sets. *significant effect of time, irrespective of group ($p < 0.01$). #significant effect of smoking status, irrespective of time ($p < 0.01$).

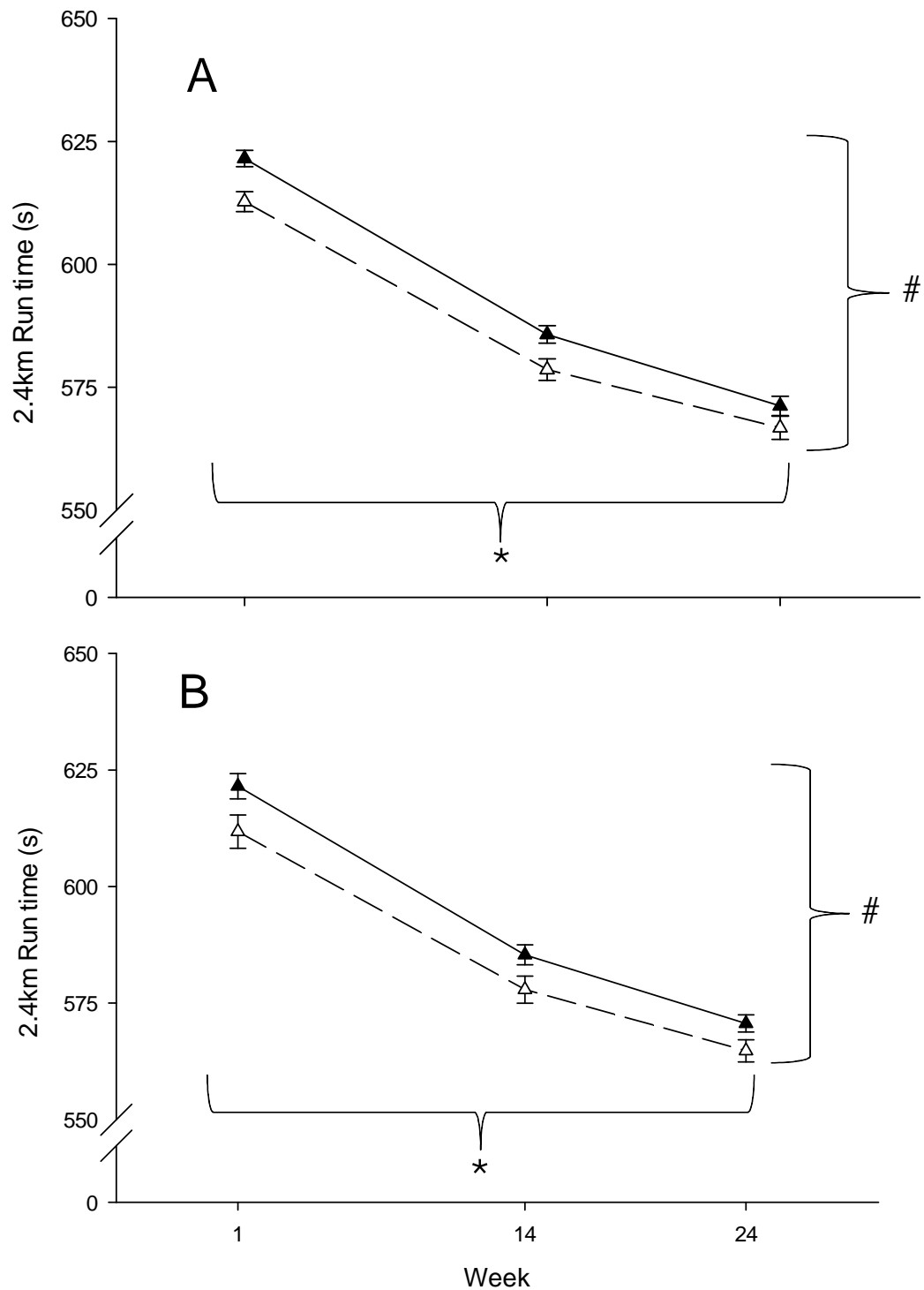


Fig 7.3. 2.4km run performance in groups NS (Open triangle) and S (Closed triangle) at weeks 1, 14 and 24 of training in all participants. A: Values are estimated marginal means with standard error bars for all participants. B: Values are means with standard error bars for all participants with complete data sets *significant effect of time, irrespective of group ($p < 0.01$). #significant effect of smoking status, irrespective of time ($p < 0.01$).

7.4 Discussion

The present study was designed to determine whether habitual smoking affected development of physical fitness during 24 weeks of initial military training. Training elicited a similar significant improvement in press up, sit up and 2.4 km run performance in both smokers and non-smokers. Physical performance data analysed by linear mixed model using all participants were similar to when solely including those who completed training. As such, cigarette smoking did not appear to impact on the development of physical fitness. However, trainees who smoked were significantly less fit than non-smoking trainees throughout the duration of training.

It has been postulated that smoking can attenuate the ability to develop physical fitness during long term training (Hoad & Clay 1992). However, no difference in the improvement in physical performance between non-smokers and smokers in the present study suggests that habitual smoking did not impair the rate of development of physical fitness in this population. Similarly, habitual smoking did not influence performance improvement or muscular adaptation in 14 weeks of British infantry training (Chapter 6). This was observed alongside chronically elevated markers of oxidative stress and inflammation in habitual smokers, which have previously been suggested as possible mediators for impaired adaptation to exercise training in smokers. These studies give novel evidence for there being no adverse effect of smoking on the progression of physical fitness during training.

Although improvement in performance in smokers was not significantly smaller than that of non-smokers, it is interesting to note that on average smokers performed significantly more poorly in all parameters in both analyses. In agreement with the majority of current research, this supports the association between habitual smoking and lower overall physical fitness in comparison to non-smokers (Zadoo et al. 1993; Benaards et al. 2003). Participation in fewer health promoting behaviours in smokers is proposed as having an impact on their lower physical fitness (Larson et al. 2007), and could explain the difference between smokers and non-smokers at entry to training. Additionally, habitual smoking can have effects on the cardiorespiratory system that can adversely affect exercise performance, such as reduced lung function (De & Tripathi 1988), cardiac response

to exercise (Mendonca et al. 2011) or increased blood pressure (Al-Safi 2005). The current study is the first, however, to suggest an association between smoking and reduced performance in muscular endurance tasks. Alongside factors already discussed, smokers exhibit impaired microcirculation (Siafaka et al. 2007) and lower fatigue resistance (Morse et al. 2007; Morse et al. 2008; Wüst et al. 2008) within muscles, which could be detrimental to performance in these tasks.

Linear mixed model analysis of physical performance data using the entire data set presents similar findings to when only including those individuals that completed training. Although the linear mixed model attempts to correct for missing data, the trainees in the latter stages of training are those that have adapted more positively to training, and could give an unintentionally biased sample. However, identical significant group and training effects existed in both analyses, providing confidence that the modelling approach was suitably robust even with some incomplete datasets. Therefore, while it is still possible there would be a greater adverse influence of smoking on performance improvement in those discharged from training the present study suggests this was unlikely to have markedly affected training outcome.

Some limitations concerned with military training may have also limited the observation of different adaptive responses between groups. Military training is designed to prepare trainees for the physically demanding roles necessary to be an effective soldier. This is supported in the current study by significant temporal improvements in all fitness parameters, irrespective of group. As such, the effectiveness of the progression of physical training may be such that any deleterious effect of smoking is too small to be measurable by comparison. Additionally, given trainees have knowledge of pass criteria in Army physical fitness tests, fitter individuals may not perform maximally if the successful completion of the test is assured. Alternatively, the opportunity for fitter individuals to improve performance may be hindered in this training environment. With an aim for all trainees to reach comparable fitness, a large proportion of military physical tasks are completed as a group at a set pace, where trainees work at different intensities relative to their own absolute fitness. For non-smokers this has negative implications given the evidence supporting typically higher physical

fitness than smokers (Larson et al. 2007). These factors suggest results from these tests may not be a true reflection of the extent of adaptation to training in the cohort.

The performance parameters measured in the current study are used by the military as a fitness indicator of military readiness relative to age and gender. Yet the absolute size of the discrepancy observed between smokers and non-smokers is small, such that the average performance of smokers in the present study would still be sufficient to pass military physical fitness tests. As such, the adverse influence of habitual smoking on physical fitness is unlikely to markedly affect operational effectiveness.

The current study has shown that while British infantry initial training significantly improves performance in physical tasks, habitual smoking can potentially impair physical fitness. There is, however, no evidence for an impact of habitual smoking on improvement in performance during long-term exercise training. Similar performance improvement was found when using a modelled approach based on data from all trainees to those trainees who completed training, which supports that sample bias from trainee drop-out was not a substantial confounder to study validity. Despite effects potentially being lessened by the limited nature of the military physical tests administered, smoking-induced differences in physical performance did not greatly affect attainment of military performance goals. As such, habitual smoking in this population is unlikely to have considerable impact on operational effectiveness solely based on physical fitness. However, the effects on physical fitness observed in this study do highlight potential adverse effects on health of substantial tobacco exposure in military training populations.

CHAPTER 8

Study 5

The Effect of Smoking Status on the Acute Responses of Markers of Inflammation and Hormones to Military Exercise

8.1 Introduction

Both habitual smoking and arduous military field training have been observed to have marked effect on concentrations of circulatory hormones and immune markers (Andelid et al. 2007; Steptoe & Ussher 2006; Nindl, Barnes, et al. 2007; Tanskanen, Kyröläinen, et al. 2011). However, it is unclear whether smokers respond differently from non-smokers to these intense periods of military exercise. This is particularly relevant given that in military training populations, smoking is highly prevalent (Heir & Eide 1997; Klesges et al. 2001) and is associated with reduced physical fitness (Zadoc et al. 1993; Haddock et al. 2007) and poorer training outcome (Marti et al. 1988; Reynolds et al. 1999; Knapik, Sharp, et al. 2001). Examining resting markers of inflammation and endocrine status both at entry (Chapter 5) and during ten weeks of initial military training (Chapter 6) have shown few differences aside from elevated C-reactive protein (CRP) in smokers compared with non-smokers.

Exercise evokes a transient elevation in inflammatory cytokines and alterations in hormone secretion into the circulation. In response to acute exercise, skeletal muscle releases interleukin (IL)-6 into the circulation which is thought to be responsible for the subsequent stimulation of both anti- and pro-inflammatory cytokines (Petersen & Pedersen 2005), and the increase in CRP in the hours after exercise (Wilund 2007). The predominance of studies have observed that as an immediate response to exercise circulating insulin-like growth factor (IGF)-1 remains relatively unchanged (Meckel et al. 2009; Stokes et al. 2010; Wahl et al. 2010) but basal levels significantly reduce in response to chronic exercise, military field exercise or intensive periods of overtraining (Nindl et al. 2003; Nindl & Pierce 2010). Typically, the testosterone/cortisol ratio increases acutely following exercise as characterised by elevations in testosterone and either relatively smaller increases or unchanged concentrations of cortisol (Hayes et al. 2010; Wahl et al. 2010;

Hansen et al. 2012), before returning to pre-exercise values within an hour (Daly et al. 2005; Fry & Lohnes 2010).

Currently, the hormonal and inflammatory responses of habitual smokers to singular or multiple bouts of exercise are not well described. To our knowledge, only the response of oxidative stress to exercise has been examined in smokers and non-smokers, reporting a proportionally greater response in smokers to maximal graded exercise (Bloomer et al. 2007; Gochman et al. 2007; El Abed et al. 2011). Despite purported links to oxidative stress, neither inflammatory nor hormonal factors have been examined in habitual smokers and non-smokers in response to exercise. It may be that different acute responses of hormones and inflammatory markers to arduous training stresses in smokers may indicate whether smokers experience greater physiological strain during military exercise on consecutive days.

This study examined the acute responses of hormones and markers of inflammation to arduous bouts of military exercise on two consecutive days in habitual smokers and non-smokers. The study objectives were to examine whether the responses of biochemical parameters to exercise or to two days of simulated operational stress would differ between smokers and non-smokers.

8.2 Methods

8.2.1 Participants

Thirty five British Army parachute regiment trainees (age 22 ± 3 yr; mass 76.9 ± 8.0 kg; height 178 ± 6.0 cm) undertaking the Pegasus Company (P Company) selection week took part in the study.

8.2.2 P Company week

P Company week takes place at the end of the 26 week parachute regiment training course at ITC(C). It is designed to assess trainee readiness to join the

parachute regiment by examining performance in a selection of arduous physical tasks simulating operational stress and testing various components of muscular and endurance fitness.

8.2.3 Study protocol

The study was a pre-post repeated measures independent group comparison with two exercise bouts. The study took place over two consecutive days of P Company testing week, containing a military exercise task on each morning; the “10-miler” on day 1 and the “log race” on day 2. The 10-miler required trainees, as a platoon, to cover 10 miles of varying terrain within 1 hour and 50 minutes with each trainee carrying a pack weighing 33 lbs (15 kg). The log race required trainees in groups of 6-8 to carry a 120 kg log over approximately 2 miles of varying terrain in as short a time as possible within 18 minutes. Blood samples were taken on waking (0500-0600) prior to the 10-miler (waking pre-10-miler) and following the 10-miler (post-10-miler) on day 1, and on waking prior (0500-0600) to the log race (waking pre-log race) and immediately following log race (post-log race) on day 2. Both events started at approximately 0900 after the participants had consumed breakfast.

8.2.4 Anthropometric data

Body mass, height and estimated body fat percentage were measured on the night prior to the beginning of data collection.

8.2.5 Blood biochemistry analysis

Blood samples were analysed for concentrations of CRP, IL-6, testosterone, cortisol and IGF-1.

8.2.6 Statistical analyses

Statistical analyses were performed using SPSS software (SPSS for Windows: Version 16.0). Initially, an independent t-test was performed on baseline anthropometric data to test for any initial differences between groups. As the study design is a repeated measures independent group comparison with one unpaired (smoking) and one paired (time) independent variable all biochemical variables were analysed by two-way mixed model analysis of variance (ANOVA). Post-hoc analysis with bonferroni adjustment was used to determine the location of variance in the event of a significant interaction or training effect. Tests for normality were not performed as it is established that when analysing physiological data an ANOVA is robust to skews to the mean (Maxwell & Delaney, 1990). An alpha level of $p \leq 0.05$ was used to define the statistical significance of within- or between-participant effects. Greenhouse Geisser ANOVA outputs were used in cases where Mauchley's test of sphericity showed data to be aspherical. Population characteristics are presented as mean \pm SD. Biochemical data are presented as mean \pm SE.

8.3 Results

8.3.1 Participants

Participant characteristics and anthropometric data organised by group are presented in Table 8.1. The non-smoking and smoking groups comprised 20 and 15 trainees, respectively. The smoking group had a mean (\pm SD) cigarette consumption of 11.8 (\pm 5.3) per day for an average of 7.0 (\pm 2.8) years. Independent t-test results found no significant differences in anthropometric data between groups at baseline ($p > 0.05$).

Table 8.1. Participant characteristics by group. Values are means \pm SD.

Variable	Smoking Status		
	Smokers (n=15)	Non-smokers (n=20)	All (n=35)
Age (yr)	22 \pm 3	22 \pm 3	22 \pm 3
Body Mass (kg)	75.9 \pm 6.9	77.8 \pm 8.9	76.9 \pm 8.0
Height (m)	1.77 \pm 0.05	1.78 \pm 0.07	1.78 \pm 0.06
Body Fat (%)	12.7 \pm 2.1 (n=13)	14.2 \pm 2.7 (n=18)	13.6 \pm 2.6

8.3.2 Inflammatory markers

Serum CRP concentrations were not different between smokers and non-smokers ($p > 0.05$, Figure 8.1), but there was a significant effect of time ($p < 0.001$). Post hoc analyses showed that CRP concentrations were significantly higher at both time points on the second day (waking-pre- and post- log race) than on the first (waking-pre- and post-10-miler; $p < 0.001$). Serum IL-6 concentrations did not significantly differ between groups ($p > 0.05$, Figure 8.2). In contrast to CRP, IL-6 concentrations increased in response to exercise, with post exercise values (post-10-miler and post-log race) significantly higher than their respective pre-exercise resting values (pre-10-miler and pre-log race; $p < 0.001$). Additionally, IL-6 concentrations immediately after the 10 miler were significantly higher than after the log race. ANOVA did not identify any significant interaction effects in either inflammatory marker ($p > 0.05$).

8.3.3 Endocrine markers

Neither serum testosterone nor cortisol concentrations were different between groups ($p > 0.05$, Figure 8.3). Both markers demonstrated a significant effect of time ($p < 0.001$), and post hoc analyses showed that both exercise bouts resulted in a significant increase in cortisol and decrease in testosterone concentrations. A significant effect of time irrespective of group was evident on the

ratio of testosterone to cortisol, reducing in response to both exercise bouts ($p < 0.05$, Figure 8.4). No significant overall effects of smoking status or interaction effects were present in IGF-1 ($p > 0.05$, Figure 8.5). A non-significant trend existed for a reduction in IGF-1 concentrations over time ($p = 0.055$).

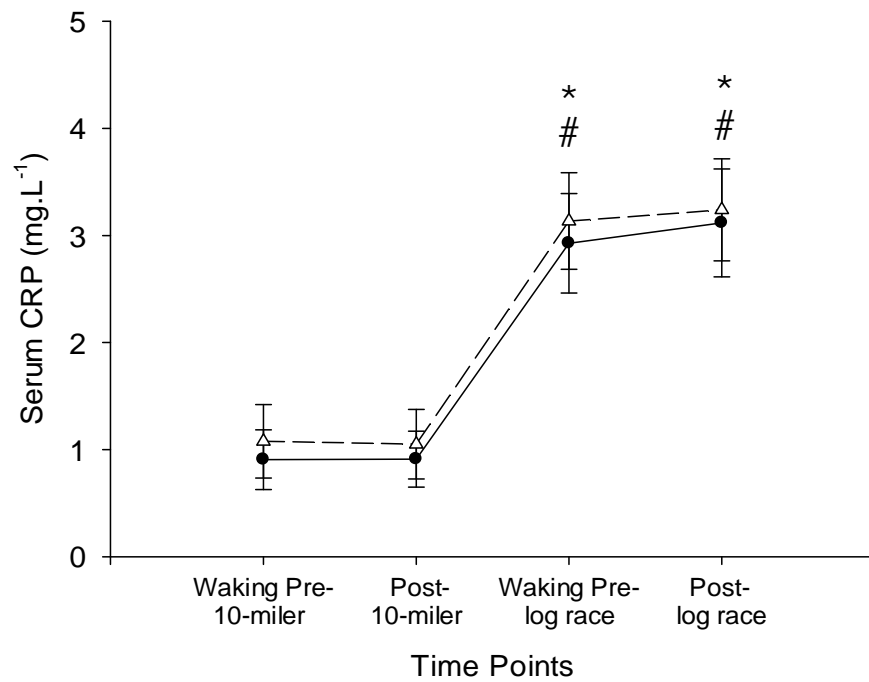


Fig 8.1. Mean serum concentration of CRP ($\text{mg}\cdot\text{L}^{-1}$) between NS (Open triangle) and S (Closed circle), across time points. Values are means with standard error bars. Time effects ($p \leq 0.05$) irrespective of group: *significant difference from waking pre-10-miler. #significant difference from post-10-miler.

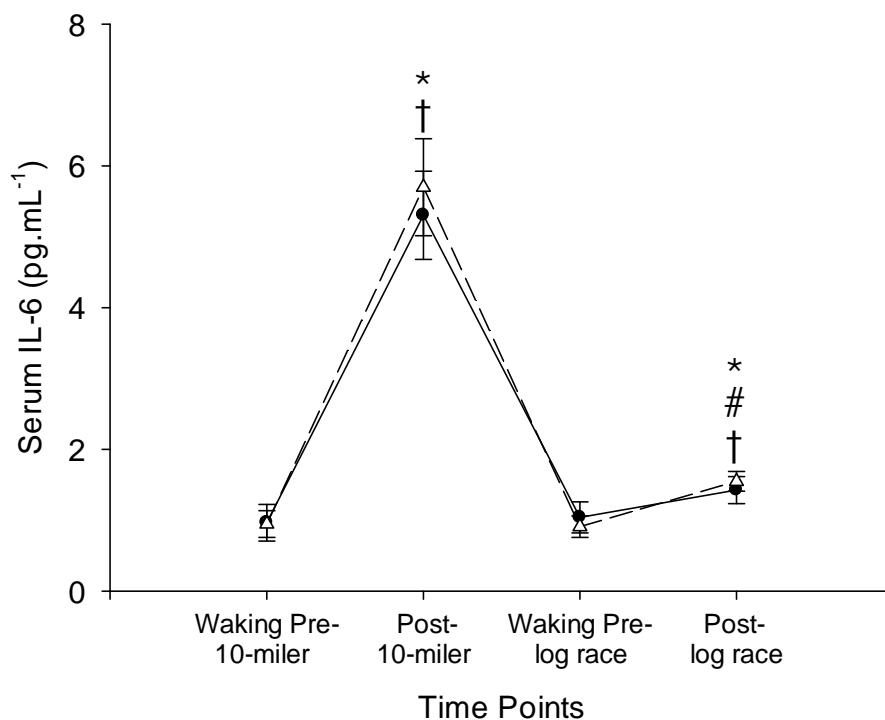


Fig 8.2. Mean serum concentration of IL-6 ($\text{pg}\cdot\text{mL}^{-1}$) between NS (Open triangle) and S (Closed circle) across time points. Values are means with standard error bars. Time effects ($p \leq 0.05$) irrespective of group: *significant difference from waking pre-10-miler. #significant difference from post-10-miler. †significant difference from waking-pre-log race.

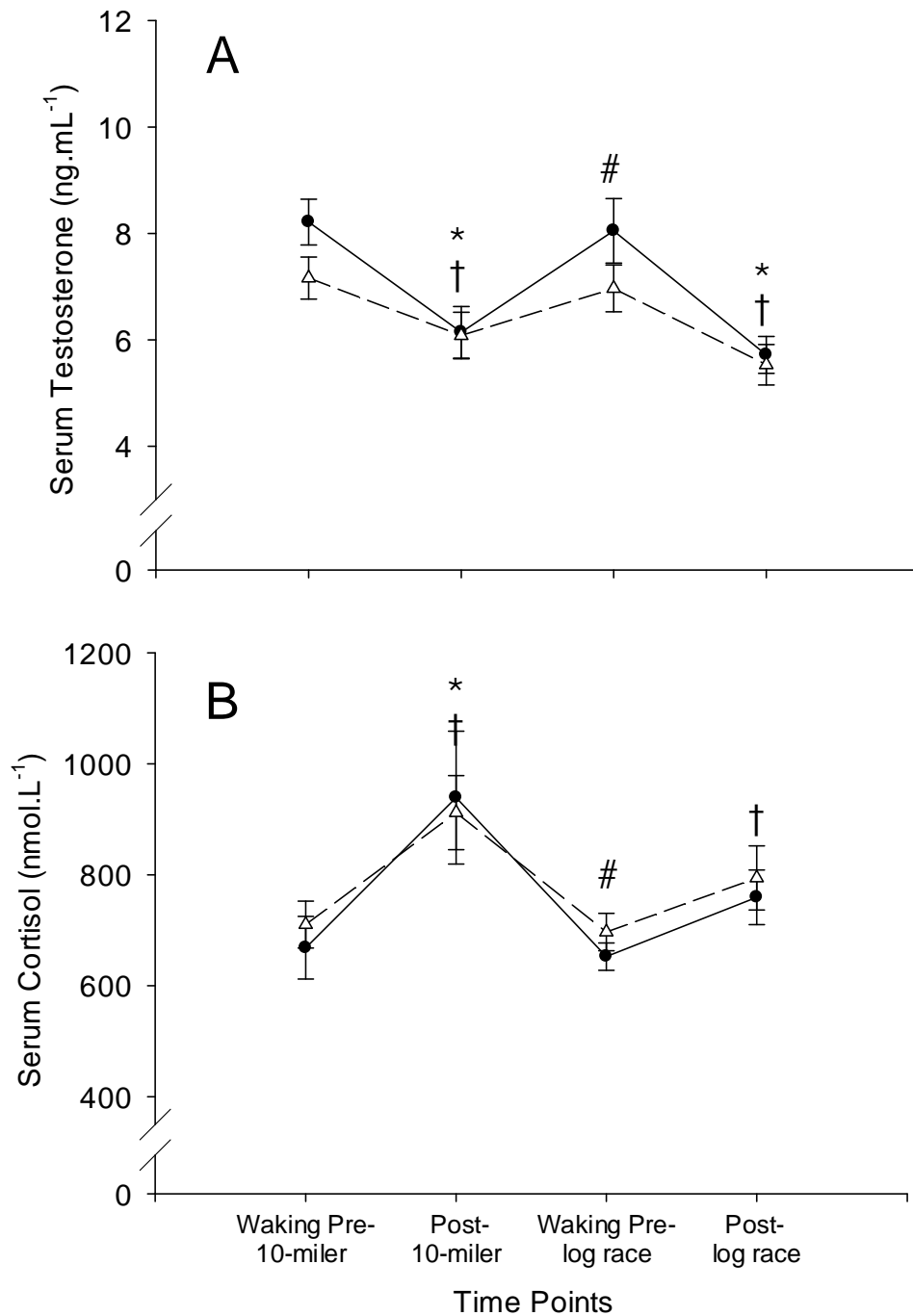


Fig 8.3. Mean serum concentrations of Testosterone (A; ng·mL⁻¹) and Cortisol (B; nmol·L⁻¹) between NS (Open triangle) and S (Closed circle) across time points. Values are means with standard error bars. Time effects ($p \leq 0.05$) irrespective of group: *significant difference from waking pre-10-miler. #significant difference from post-10-miler. †significant difference from waking-pre-log race.

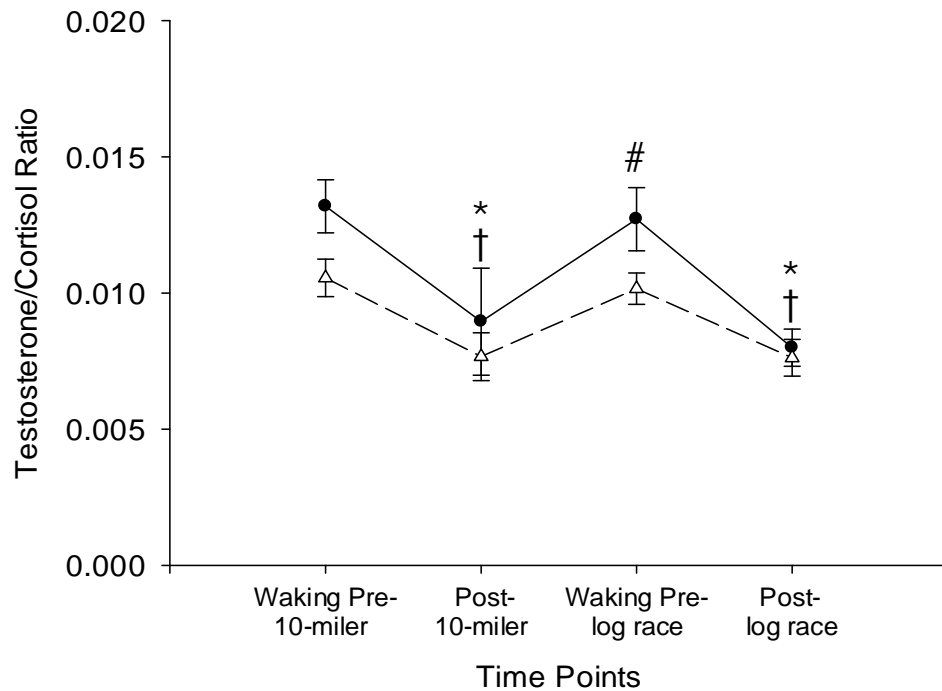


Fig 8.4. Mean serum concentrations of testosterone to cortisol ratio between NS (Open triangle) and S (Closed circle) across time points. Values are means with standard error bars. Time effects ($p \leq 0.05$) irrespective of group: *significant difference from waking pre-10-miler. #significant difference from post-10-miler. †significant difference from waking-pre-log race.

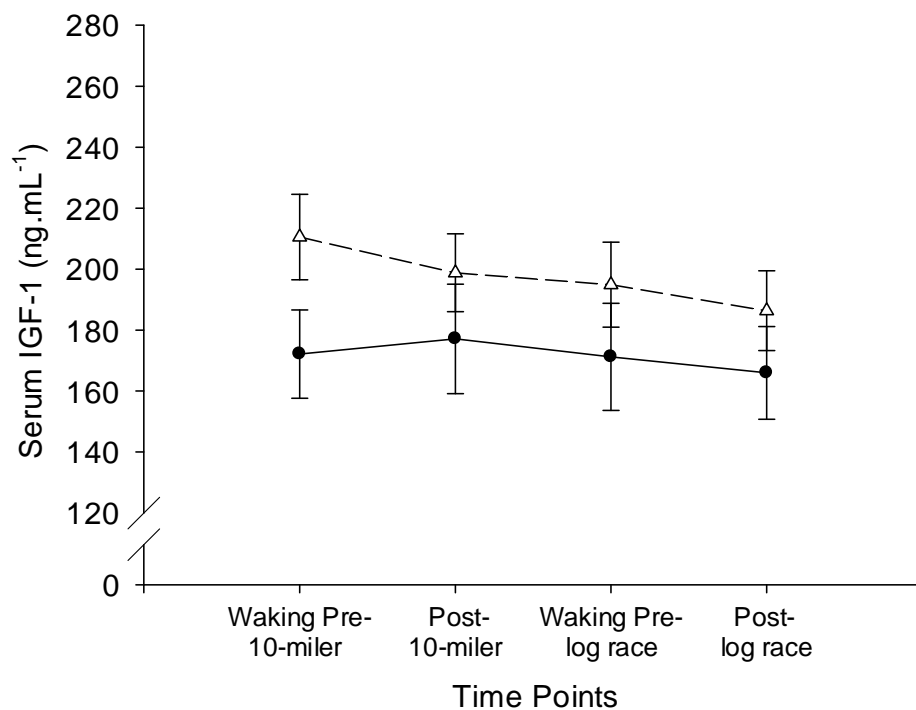


Fig 8.5. Mean serum concentration of IGF-1 ($\text{ng} \cdot \text{mL}^{-1}$) between NS (Open triangle) and S (Closed circle) across time points. Values are means with standard error bars. Non-significant trend for an effect of time ($p=0.055$).

8.4 Discussion

We examined inflammatory and endocrine markers in smokers and non-smokers both at rest and after intense bouts of exercise during two days of arduous military training. The responses of these markers to a period of simulated operational stress could reflect the extent of physiological strain. The results indicate that both bouts of exercise elicited acute increases in IL-6 and cortisol, acute decreases in testosterone, an elevation in CRP evident at rest the day following exercise, and a general decline of IGF-1 over the two-day period. However, none of the responses were different between smokers and non-smokers.

We are not aware of any study that has examined the responses of hormonal and inflammatory markers to exercise in smokers and non-smokers. The main finding of the current study is that smokers and non-smokers did not respond differently to the log race and 10-miler in any of the markers measured. Previously, the oxidative stress response to maximal graded cycling exercise has been compared in smokers and non-smokers, with an exacerbated response reported in smokers (Bloomer et al. 2007; Gochman et al. 2007). Given that both acute and chronic systemic inflammation in smokers is associated with oxidative stress (Van der Vaart et al. 2004; Helmersson et al. 2005; Yanbaeva et al. 2007), and oxidative stress response is greater in smokers, it was hypothesised that acute inflammatory responses to exercise would also be greater in smokers than non-smokers. The fact that the inflammatory responses were not different suggests that, in this population during intense training, the effect of habitual smoking on inflammation is comparatively smaller than that elicited by exercise. It should be noted that trainees who participated in this study were nearing the end of the 26 week training course. As such, the chronic anti-inflammatory effect of long term exercise training (Plaisance & Grandjean 2006) may have counteracted the elevated CRP we observed in smokers during the early stages of training (Chapter 6), resulting in a similar inflammatory response to non-smokers.

Military field exercise, involving consecutive days of arduous training, has been shown to elicit alterations in hormone concentrations. Specifically, suppression of IGF-1 and testosterone, alongside increased circulatory cortisol have been demonstrated during periods of intensive military training (Nindl, Barnes, et

al. 2007; Kyröläinen et al. 2008). During prolonged training, increases in cortisol in particular correlate with daily and weekly training volume (Purge, Jürimäe & Jürimäe 2006; Tyyskä et al. 2010) and sleep disruption (Booth et al. 2006). As such, previous research suggests military field exercise potentially evokes a period of metabolic stress and an ostensibly catabolic state. In support of this, a trend for a decline in IGF-1 from rest on day one, to post-exercise on day two approached significance ($p=0.055$). These findings could indicate that continuation of military training of this nature would incite a state of physiological strain, similar to that of energy deficit or overtraining.

Changes in the testosterone-cortisol ratio to both bouts of exercise were similar, with increases in cortisol and reductions in testosterone. Typically, exercise induces secretion of purportedly anabolic (e.g. testosterone) and catabolic (e.g. cortisol) hormones (Spiering et al. 2009; Hayes et al. 2010; Vingren et al. 2010). It is perhaps unexpected therefore, that testosterone concentrations were lower after exercise than at rest. This finding is likely explained by the resting blood sample being taken upon waking rather than immediately before exercise. The circadian rhythm of testosterone means it is possible the resting sample reflects early morning peak concentrations (Hayes et al. 2010), against which post-exercise concentrations appear substantially reduced. Additionally, given that resistance (Wilkinson et al. 2006; Fry & Lohnes 2010; Hansen et al. 2012) and endurance exercise (Daly et al. 2005; Cadore et al. 2012; Hansen et al. 2012) have previously shown opposing testosterone responses, the combination of load carriage and aerobic exercise involved in these military tasks may demonstrate an entirely different physical challenge from previous research.

Given the lack of extant literature, it was unknown whether hormone responses to exercise would differ between smokers and non-smokers. Numerous mechanisms linked to the actions of nicotine and immune-inflammatory signalling have been suggested to explain alterations in resting hormone levels in smokers (Kirschbaum et al. 1994; Steptoe & Ussher 2006). In the present study, the responses of cortisol, testosterone and IGF-1 to military exercise were not different between smokers and non-smokers.

Current literature agrees that IL-6 increases transiently as a result of exercise, supporting the observation of significant increases in IL-6 above baseline following both exercise bouts. Additionally, the magnitude of this response is affected by exercise intensity and duration (Petersen & Pedersen 2005). The response of IL-6 to the 10-miler was significantly greater than to the log race, suggesting that the longer duration (1 hour 50 mins instead of <20 mins) of the 10-miler had a greater effect on the magnitude of the inflammatory response than the higher intensity of the log race.

Exercise on the first day caused significantly elevated resting levels of CRP on the morning of the second day, while there was no acute effect (i.e., pre- to post-exercise) of exercise on CRP. It is well-established that the rise in CRP associated with exercise can continue to develop over 24 hours (Plaisance & Grandjean 2006; Mathur & Pedersen 2008), explaining the delayed exercise-induced increase in CRP observed in the current study. The rise in resting inflammation elicited by the 10-miler has implications for consecutive days of arduous exercise without sufficient recovery. In the current study, the log race was initiated while resting CRP was elevated from exercise on the previous day to a level greater than $3 \text{ mg}\cdot\text{L}^{-1}$, and higher than normal for a population of this age and physical fitness. These data suggest CRP levels on the day following the log race could have risen further, albeit following a comparatively lower stimulation from IL-6. These findings indicate that continual repetition of consecutive days of particularly arduous exercise without sufficient recovery could induce substantial inflammation even in a young population of high physical fitness. Taking this further, we were interested to know whether the response to exercise on the second day would be different in smokers compared with non-smokers, given that this was superimposed on the challenges of the previous day. However, the responses were not different between groups.

This field study investigated inflammatory and endocrine responses to consecutive days of military exercise in a real-world setting, and how these might differ between smokers and non-smokers. Habitual smoking did not have a significant effect on markers of inflammation or hormones during the two day training period. Previous laboratory studies have demonstrated differing responses of oxidative stress markers to exercise in smokers compared with non-smokers (Bloomer et al. 2007), and it is possible that with the greater experimental control

afforded by a laboratory setting, the findings of the present study might have been different. Similarly, including an immediately pre-exercise blood sample would have allowed a clearer differentiation between the exercise-induced changes in inflammatory markers and hormones from the effects of circadian rhythms and meals consumed between samples. However, the responses examined are an externally valid and accurate representation of exercise in the military, whereby the absence of differences between smokers and non-smokers challenge the relevance of a laboratory study. It is clear that the nature of exercise performed during this study had a profound effect on markers that indicate physiological strain similar to energy deficit and overtraining. These responses did not differ between smokers and non-smokers, indicating that habitual smoking did not influence the physiological strain experience by trainees during a two-day military exercise period.

CHAPTER 9

Study 6

The Influence of Smoking Status on Injury Incidence in British Infantry Initial Training

9.1 Introduction

Arduous physical training in a military setting is associated with a high incidence of musculoskeletal and overuse injuries (Knapik, Canham-Chervak, et al. 2001), especially during initial training (Blacker et al. 2005). Injuries to trainees are costly, both to the individual and the military organisation, due to the potential loss of training time, long and expensive rehabilitation and, in some severe cases, medical discharge from service. In an attempt to reduce injury risk, research has been carried out to describe injury incidence (Kaufman et al. 2000; Knapik, Canham-Chervak, et al. 2001), identify risk factors for training injuries (Altarac et al. 2000; Knapik, Sharp, et al. 2001; Blacker et al. 2008) and develop interventions for the prevention of injury (Knapik et al. 2004; Bullock et al. 2010) within military populations.

Cigarette smoking is the most widely identified independent risk factor for training-related injury in military populations (Reynolds et al. 1999; Altarac et al. 2000; Knapik, Sharp, et al. 2001; Etherington & Owen 2002). It has been reported that during Royal Marine training, the relative risk of training-related injury for habitual cigarette smokers was 1.7 times that of non-smoking counterparts (Munnoch & Bridger 2007). Additionally, a dose-response association has been suggested, where risk of injury increases with cigarette consumption rate (Knapik, Sharp, et al. 2001). Numerous mechanisms for heightened injury risk in habitual smokers have been postulated, including lower physical fitness (Kobayashi et al. 2004), physical activity (Conway & Cronan 1992) and nutritional intake (Klesges et al. 1990); increased risk-taking behaviour (Zuckerman & Kuhlman 2000); impaired immune function (Arcavi & Benowitz 2004), recovery (Sherwin & Gastwirth 1990) and bone health (Wong et al. 2007); and alterations in oxidative and inflammatory processes (Cross et al. 1998; van der Vaart et al. 2004).

Previous research into injury incidence is abundant in United States (US) and Scandinavian military populations, demonstrating values as high as 51% in US

infantry (Knapik et al. 1993), 40% in US marines (Almeida et al. 1999), 24% in Norwegian basic training (Heir & Eide 1997) and 32% in Royal Norwegian Navy personnel (Morken et al. 2007). In British training establishments incidence has been reported to range from 4% (Greeves 2006) to 26.5% (Blacker et al. 2005). Considerable variation in injury incidence might be a result of differences in training environment, duration and location in addition to methodological differences between studies. Within British Army training establishments both the highest medical discharge rate (Blacker et al. 2005) and lowest first-time pass rate (40-50%; Carter et al. 2006) have been reported at infantry training centre (Catterick) (ITC(C)). Despite this, previous studies at ITC(C) have not quantified more than medical discharge rates, warranting a more comprehensive study of injury in this population

Injuries most commonly reported in military populations are musculoskeletal overuse injuries predominantly in the knee and lower leg (Ross 1993; Kaufman et al. 2000). In British Army recruit training, injuries to the back, foot and lower leg were the most common, comprising between 50% (Greeves 2006) and 70% (Wilkinson et al. 2011) of all training injuries. Specifically, high incidence of lower back pain, shin splints, and overuse knee injuries such as patella-femoral syndrome, patellar tendinitis and iliotibial band syndrome have been reported (Jones et al. 1993; Kaufman et al. 2000). Injuries of this nature are highly indicative of the physical stresses produced by repetitive exhaustive load-bearing exercise common to military training, and largely affecting the lower-limb and supporting musculature.

The aim of this study was to examine overall injury incidence and prevalence of training-related injuries specifically to the lower-limb and lumbar spine in the British infantry training population at ITC(C), and to investigate whether habitual smokers are at greater risk of training-related injury than non-smokers.

9.2 Methods

9.2.1 Participants

Questionnaire data were collected in week 1 of training from a cohort of male infantry trainees.

9.2.2 Study design

Volunteers were asked to complete the Military Pre-training Questionnaire (MPQ) at entry to training to determine individual smoking characteristics. Lower limb and lumbar spine injury data were collected, retrospectively, from military medical records. Injuries were organised by those that occurred from training, those that resulted in training time-loss and those that had acute or chronic causes. Anatomical location and injury type were also recorded. Injury incidence and clinical injury incidence were calculated for non-smokers and smokers, as well as smokers organised by cigarette consumption. To compare groups, relative risk and odds ratio were calculated for smokers with non-smokers acting as a control group. Entry and exit dates from military databases, alongside time lost to injury, were used to determine average injury severity and total training exposure of the population. Total training exposure allowed quantification of incidence rate and average burden of injuries in 1000 trainee-days. Rate ratios were calculated to compare incidence rate and burden of injuries between smoking and non-smoking groups. The proportion and severity of separate injury types were also calculated.

9.2.3 Statistical analyses

Odds and rate ratios were used to compare injury incidence and incidence rate, respectively, between non-smokers and smoking groups. Differences between groups from odds ratio and rate ratio were determined by calculated z-scores.

$$z = \frac{\log RR}{SE_{(\log RR)}}$$

Where RR can be substituted by any ratio value.

Subsequently, two-tailed P values were calculated from the z scores such that $p < 0.05$ would signify a significant difference between non-smokers and smokers, as well as smoking subgroups. Injury incidence and injury proportion are presented as percentages. Injury incidence rate is presented per 1000 trainee-days and average injury severity is median days lost to injury with interquartile range (IQR). Relative risk, odds and rate ratio are present with 95% confidence intervals.

9.3 Results

In total, 1972 trainees completed the present study. Sufficient medical data were available for 1810 trainees to be used in analysis of injury incidence. Injury data in this study refer to injury to the lower limb and lumbar spine. All-cause injury incidence to the lower limb and lumbar spine in initial training was 63%, where trainees who sustained one or more injury of any kind to these locations totalled 1142. A total of 1045 of these were attributable to training, representing a training-related injury incidence of 58% (Table 9.1).

A total of 1682 trainees had sufficient training data to calculate exposure time and were included in analysis of clinical incidence and incidence rate. This sample showed that 0.98 training injuries and 0.57 time-loss training injuries were sustained per trainee during initial training. Median (IQR) severity of time-loss training injury was 14 (43) training days lost per injury. Burden calculations showed that time-loss training injuries resulted in 123 training days lost per 1000 trainee-days.

Table 9.1. Training injury incidence (trainees with one of more training-related injury within trainees at risk), organised by injury classification (n=1810).

Injury Classification	Trainees Injured	Incidence (%)
<i>Training-related</i>	<i>1045</i>	<i>58</i>
Time-loss	583	32
Acute time-loss	216	12
Overuse time-loss	367	20

9.3.1 Injury incidence in non-smokers and smokers

From the 1810 trainees, 595 were non-smokers and 893 were regular smokers, who averaged (mean (\pm SD)) 11.7 (\pm 5.7) cig/day for 6.0 (\pm 3.2) years. Relative risk of sustaining a training injury in smokers was 1.09 (0.99-1.19) that of non-smokers. Odds ratios demonstrated that the incidence of training injury (95% CI) in smokers (60 (57-63)%) was significantly higher than that in non-smokers (55 (51-59)%; $p < 0.01$). Furthermore, incidence of both time-loss training injuries and, more specifically, time-loss overuse injuries were significantly greater in smokers (Table 9.2; $p < 0.01$). The risk of sustaining acute time-loss training injuries was not significantly different between smokers and non-smokers.

Compared with non-smokers, groupings of smokers by cigarette consumption exhibited significantly higher risk of training-related injuries and of overuse time-loss training injuries. With the exception of the moderate smoking group, higher risk of time-loss training injury existed in smoking subgroups.

9.3.2 Injury incidence rate in non-smokers and smokers

The overall incidence rate for training injuries was 5.95 (5.66-6.24) injuries per 1000 trainee-days. Incidence rates of both training-related injuries and time-loss training injuries were significantly higher in smokers ($p < 0.02$) than non-smokers (Table 9.3). Median (IQR) severity of time-loss training injury was identical in non-smokers (14 (38)) and smokers (14 (44)). Burden (CI 95%) calculations, however, showed that the 132 (120-144) training days lost per 1000 trainee days from time-loss training injury in smokers was significantly higher than the 104 (92-116) days lost in non-smokers ($p < 0.01$).

Significantly higher incidence rates of training and time-loss training injuries were observed in light smokers when compared to non-smokers ($p < 0.05$), but were not evident in moderate and heavy smokers. Neither acute nor overuse time-loss injury incidence rate were significantly different in smokers and smoking groups from non-smokers.

Table 9.2. Injury incidence, relative risk and odds ratio among training injuries organised by smoking group (n=1810).

Injury Classification	Incidence (95% CI)	Relative Risk (95% CI)	Odds Ratio (95% CI)
<i>Injured in Training</i>	58 (56-60)		
Non-smokers	55 (51-59)	-	-
Smokers	60 (57-63)	1.09 (0.99-1.19)	1.22 (1.12-1.34)*
<i>Light smokers</i>	59 (53-65)	1.07 (0.94-1.21)	1.17 (1.03-1.33)*
<i>Moderate smokers</i>	60 (56-64)	1.09 (0.98-1.20)	1.22 (1.10-1.35)*
<i>Heavy smokers</i>	62 (54-70)	1.12 (0.97-1.31)	1.33 (1.14-1.54)*
<i>Time-loss</i>	32 (30-34)		
Non-smokers	30 (26-34)	-	-
Smokers	34 (31-38)	1.15 (0.99-1.34)	1.23 (1.06-1.44)*
<i>Light smokers</i>	37 (31-43)	1.22 (1.00-1.50)	1.35 (1.10-1.66)*
<i>Moderate smokers</i>	33 (29-37)	1.11 (0.93-1.32)	1.16 (0.98-1.38)
<i>Heavy smokers</i>	36 (28-44)	1.20 (0.93-1.55)	1.31 (1.01-1.69)*
<i>Time-loss Acute</i>	12 (10-13)		
Non-smokers	13 (10-16)	-	-
Smokers	13 (11-15)	0.97 (0.74-1.26)	0.96 (0.73-1.26)
<i>Light smokers</i>	13 (9-18)	1.02 (0.70-1.49)	1.03 (0.70-1.50)
<i>Moderate smokers</i>	13 (10-15)	0.96 (0.70-1.30)	0.95 (0.70-1.30)
<i>Heavy smokers</i>	11 (6-17)	0.85 (0.51-1.44)	0.84 (0.50-1.41)
<i>Time-loss Overuse</i>	20 (18-22)		
Non-smokers	17 (14-20)	-	-
Smokers	22 (19-25)	1.30 (1.05-1.62)	1.38 (1.11-1.72)*
<i>Light smokers</i>	23 (18-28)	1.38 (1.03-1.84)	1.49 (1.12-1.99)*
<i>Moderate smokers</i>	21 (17-24)	1.23 (0.96-1.57)	1.28 (1.00-1.64)*
<i>Heavy smokers</i>	25 (17-32)	1.47 (1.04-2.07)	1.62 (1.14-2.29)*

*indicates that odds ratio demonstrates injury incidence is significantly different from non-smokers

Table 9.3. Incidence rate (per 1000 trainee-days) and rate ratio of injury classifications within training-related injuries by smoking group (n=1682).

Injury Classification	Incidence rate (95% CI)	Rate Ratio (95% CI)
<i>All Training Injuries</i>	5.95 (5.66-6.24)	
Non-smokers	5.44 (4.97-5.92)	-
Smokers	6.28 (5.86-6.70)	1.15 (1.09-1.22)*
<i>Light smokers</i>	6.58 (5.75-7.40)	1.21 (1.12-1.31)*
<i>Moderate smokers</i>	6.08 (5.53-6.63)	1.12 (1.05-1.19)
<i>Heavy smokers</i>	6.56 (5.40-7.71)	1.20 (1.09-1.33)
<i>Time-loss Injuries</i>	3.43 (3.21-3.64)	
Non-smokers	3.09 (2.70-3.45)	-
Smokers	3.65 (3.33-3.97)	1.18 (1.10-1.27)*
<i>Light smokers</i>	4.00 (3.36-4.64)	1.30 (1.17-1.43)*
<i>Moderate smokers</i>	3.45 (3.04-3.87)	1.12 (1.03-1.22)
<i>Heavy smokers</i>	3.75 (2.88-4.63)	1.22 (1.06-1.39)
<i>Time-loss Acute</i>	1.23 (1.10-1.37)	
Non-smokers	1.19 (0.97-1.41)	-
Smokers	1.37 (1.18-1.57)	1.15 (1.02-1.30)
<i>Light smokers</i>	1.58 (1.18-1.99)	1.33 (1.13-1.56)
<i>Moderate smokers</i>	1.30 (1.05-1.55)	1.09 (0.95-1.25)
<i>Heavy smokers</i>	1.22 (0.72-1.71)	1.02 (0.81-1.28)
<i>Time-loss Overuse</i>	2.19 (2.02-2.37)	
Non-smokers	1.90 (1.62-2.18)	-
Smokers	2.27 (2.02-2.53)	1.20 (1.09-1.32)
<i>Light smokers</i>	2.42 (1.92-2.92)	1.27 (1.12-1.45)
<i>Moderate smokers</i>	2.15 (1.83-2.48)	1.13 (1.02-1.26)
<i>Heavy smokers</i>	2.54 (1.82-3.26)	1.34 (1.14-1.58)

*indicates that rate ratio demonstrates injury incidence rate is significantly different from non-smokers

9.3.3 Injury types

The most prevalent training-related injury type sustained was non-specific soft tissue injury (51%), followed by muscle strain (12%) and blisters (12%). Similar injury types were observed for time-loss training injuries (Table 9.4). The most common anatomical sites for training-related injury were knee (25%), foot (24%), ankle (17%) and tibia/fibula (9%). As such, injuries to the lower leg contained >75% of all lower body injuries sustained in training.

Further analysis using both site and type revealed that non-specific soft tissue injuries to the knee, ankle and foot were the most common time-loss training injuries (Table 9.5). The highest median injury severity was observed in non-specific soft tissue injury for the shin (33 days lost), followed by non-fracture bone injury of the shin (30 days lost). Highest burden values were observed for soft tissue of the knee and ankle, lumbar muscle strain and non-fracture bone injury of the shin.

Table 9.6 shows the incidence rate, severity and estimated burden of time-loss training injuries that could be categorised as knee pain and medial-tibial stress syndrome, both shown to be prevalent in military populations. Of these categories, MTSS exhibited the higher severity with 28 training days lost per injury, while knee pain had the higher average injury burden, with 21 training days lost per 1000 training-days. Incidence rate of these injuries did not significantly differ between smokers and non-smokers ($p>0.05$). However, for knee pain, average days lost per 1000 training days in smokers was significantly higher (24 (19-29)) than non-smokers (17 (12-22)).

A total of 62 recurrent injuries were sustained, whereby cause, anatomical site, side of body and type were the same as a previous injury. Forty-five of these, 14 in non-smokers and 22 in regular smokers, resulted in a loss in training-time. The calculated training days lost per 1000 trainee-days from recurrent time-loss training injuries was 5.4 (5.0-5.9) for non-smokers and 6.9 (6.5-7.4) for smokers, and did not significantly differ ($p>0.05$).

Table 9.4. Proportion of types and sites of injury within training-related injuries

	Training Injuries		Time-loss Training Injuries	
	Injuries	Proportion (%)	Injuries	Proportion (%)
<i>Type</i>				
Non-specific soft tissue	899	50.5	516	29.0
Muscle strain	216	12.1	117	6.6
Blister	208	11.7	60	3.4
Non-fracture bone	81	4.6	66	3.7
Ligament	72	4.0	65	3.7
Tendon	65	3.7	42	2.4
Stress Fracture	27	1.5	27	1.5
Fracture	26	1.5	25	1.4
Laceration	21	1.2	11	0.6
NFCI	20	1.1	18	1.0
Bruising	14	0.8	1	0.1
Cartilage	5	0.3	4	0.2
<i>Anatomical Site</i>				
Knee	436	24.5	270	15.2
Foot	435	24.4	207	11.6
Ankle	308	17.3	195	11.0
Tibia/Fibula	165	9.3	110	6.2
Lumbar	135	7.6	74	4.2
Thigh	118	6.6	46	2.6
Pelvis	57	3.2	50	2.8

Table 9.5. Incidence rate (1000 trainee-days), average severity (training days lost to injury) and burden (training days lost per 1000 trainee-days) of the most prevalent time-loss training injuries.

Injury type	Site	Number of injuries	Incidence rate	Injury Severity (Median(IQR))	Burden (95% CI)
Non-specific soft tissue	Knee	225	0.81	15 (40)	26 (22-29)
Non-specific soft tissue	Ankle	112	0.40	14 (33)	13 (11-16)
Non-specific soft tissue	Foot	96	0.35	10 (35)	9 (7-11)
Muscle strain	Lumbar Spine	65	0.23	21 (45)	10 (8-12)
Blister	Foot	56	0.20	4 (6)	2 (1-2)
Ligament	Ankle	53	0.19	9 (26)	5 (4-6)
Non-fracture bone	Tibia/Fibula	50	0.18	30 (52)	9 (7-12)
Non-specific soft tissue	Tibia/Fibula	40	0.14	33 (50)	7 (5-9)

Table 9.6. Incidence rate (1000 trainee-days), average severity (training days lost to injury) and burden (training days lost per 1000 trainee-days) of key injury categories within time-loss training injuries, organised by smoking status.

Injury Category	Number of injuries	Incidence rate	Injury Severity (Median(IQR))	Burden (95% CI)
Knee pain	156	0.56	20 (40)	21 (17-24)
<i>Non-smokers</i>	43	0.46	24 (43)	17 (12-22)
<i>Smokers</i>	88	0.65	19 (33)	24 (19-29)*
Medial-tibial stress syndrome	92	0.33	28 (50)	16 (13-20)
<i>Non-smokers</i>	25	0.27	37 (43)	15 (9-21)
<i>Smokers</i>	51	0.38	26 (45)	17 (12-22)

*indicates that rate ratio demonstrates burden is significantly different from non-smokers

9.4 Discussion

The current study investigated the risk of training-related injury to the lower limb and lumbar spine in regular smokers compared with non-smokers during British infantry training. The results indicate that 58% of individuals sustained one or more training-related injury during initial training, and 32% of trainees sustained an injury that resulted in loss of training time. Trainees who smoked regularly had significantly greater risk of training-related injury, time-loss training injury and injuries specifically attributed to overuse. Moreover, average training time lost due to time-loss training injuries was greater in habitual smokers.

This study is the first to comprehensively describe incidence of training injuries in the largest training centre in the British Army. A high incidence of injury has previously been reported in military training populations (Knapik, Sharp, et al. 2001; Etherington & Owen 2002; Blacker et al. 2008), with basic military training incurring injuries in an average of 25% of trainees (Jones & Knapik 1999). Injury epidemiology in British military training populations have reported lower incidence of training injuries than the current study. Training injuries requiring referral to a remedial instructor occurred in 4% (Greeves 2006), 5.6% (Blacker et al. 2008) and 16.7% (Blacker et al. 2005) of trainees in various British Army training locations, compared with time-loss training injuries in 32% of trainees in the current study. This is particularly noteworthy given the current study focused on injuries to the lumbar spine and lower limb only, where the above studies included all anatomical sites. Training establishment attended has been shown to be a risk factor for injury (Blacker et al. 2005), which suggests that characteristics of the trainees involved or the training courses themselves, such as duration and training content, incur different degrees of injury risk. Other than greater duration, exact differences in content between the training course at the current study location and others in the British Army are not easily quantified. It is possible that those differences could explain the comparatively high injury incidence observed at ITC(C), and high medical discharge rate reported previously (Blacker et al. 2005; Carter et al. 2006).

In military training populations, previous research has identified habitual smoking as an independent risk factor for injury (Reynolds et al. 1999; Altarac et al.

2000; Knapik, Sharp, et al. 2001; Etherington & Owen 2002). Altarac et al. (2000) also reported significantly higher risk of overuse injuries in US Army basic training, in agreement with the current study. Additionally, Munnoch and Bridger (2007) observed higher relative risk of injury in Royal Marine trainees who smoked >10 cigarettes per day compared to light smokers (1-9 cig/day), showing evidence of a dose-response. In the current study, light and/or heavy smoking groups, but not moderate smokers, exhibited significantly higher incidence in certain injury types. If higher injury risk in smokers was mediated solely by the act of smoking, it would follow that injury incidence would be more pronounced with increased consumption. As such, it is likely that the explanation for greater injury risk in smokers is multifaceted, influenced, in part, by intrinsic risk factors of smokers other than smoking itself. Employing univariate analysis to examine risk associated with smoking, it is possible to demonstrate that regular smokers have higher risk of training-related injury than non-smokers, but not to identify smoking *per se* as the cause of this difference. Nevertheless, possible causal mechanisms for higher injury risk in smokers have been proposed, including lower typical physical activity (Conway & Cronan 1992; Jones et al. 2000), lower physical fitness (Kobayashi et al. 2004), greater risk taking behaviour (Zuckerman & Kuhlman 2000) and reduced recovery to injury and exercise (Arcavi & Benowitz 2004; Silverstein 1992), possibly mediated by impaired immunological responses (Sopori 2002; Gonçalves et al. 2011).

Significantly higher duty days lost per 1000 trainee-days from time-loss injury in smokers in the current study is a novel finding. As the current study and others (Altarac et al. 2000) have observed higher incidence of overuse injury in smokers, it is possible that smoking adversely affects processes of regeneration and recovery to injury over prolonged periods. Significantly higher burden from time-loss injuries in smokers supports this, as well as the absence of differences between smokers and non-smokers in either incidence or incidence rate of acute injury. Smoking has been associated with both reduced production of collagen (Jorgensen et al. 1998) and impaired bone metabolism (Wong et al. 2007), influencing long term bone mass loss, which might be a mechanism responsible for higher incidence of stress fractures and other overuse injuries in smokers (Lappe et al. 2001; Vestergaard & Mosekilde 2003). It should be noted that when expressed relative to

total training time incidence of time-loss overuse training injuries did not differ between groups, thereby not supporting the assertion that smoking impairs recovery processes that prevent overuse injuries. However, the analysis of rate ratio showed this difference approached significance ($p=0.054$), and the variation between this result and that of injury incidence could be explained by the slightly smaller sample used for analysis of incidence rate.

The current study examined only training injuries to the lumbar spine and lower limb, previously shown to represent between 70% (Wilkinson et al. 2011) and 83% (Etherington & Owen 2002) of all injuries sustained in military training, also suggesting that observed injury incidence may not have been markedly changed in the current study with the inclusion of other anatomical sites. The majority of observed training injuries were to the knee, ankle and foot. Injuries to the lumbar spine and lower limb are indicative of physical stresses from exercise commonly performed during military training. Injury categories representing both MTSS and knee pain were also assessed, given their reported prevalence in military populations (Jones et al. 1993; Kaufman et al. 2000), and clinical association of MTSS with stress fracture (Detmer 1986; Bouché & Johnson 2007). The burden associated with knee pain was one week greater in smokers, further supporting the tempered recovery increasing injury severity in smokers.

A limitation of the current study is that the analysis used does not elucidate why smokers are at greater risk of injury, or what other factors influencing injury risk may exist concurrently within the smoking group. However, few studies examining smokers have studied the potential effect of increased cigarette consumption on greater injury risk. By doing this, the current study gives evidence that increasing magnitude of smoke exposure has negligible effect on injury risk, supporting a likely multivariate reason for injury risk in smokers. Overall, the current findings are in keeping with those in the literature, finding an injury incidence in excess of 25% during initial military training, and a higher risk of time-loss and overuse injuries in smoking trainees. Differences from previous literature may be due to differences in training location as well as methodological definitions and diagnosis of injuries. Due to higher incidence of overuse injuries in smokers, and greater burden from time-loss injuries, it is possible that there is an effect of smoking on injury risk related to impairment of physiological processes mediating

recovery from exercise and injury. However, mechanistic discussion for the influence of smoking on injury risk can only be speculated from previous research, and could be explored further possibly by seeking to identify intrinsic risk factors within smoking populations and extrinsic risk factors of different Army training courses. It is clear from the current study that there is a greater incidence of injury at ITC(C) than reported for other British Army training populations, but also that injury risk and potential time lost due to injury are significantly increased in trainees who smoke regularly.

CHAPTER 10

General Discussion

General Discussion

This series of studies established several differences between habitual smokers and non-smokers within a scarcely examined military training population, and observed potential influences of smoking on selected biochemical markers both at entry and during initial military training. While the health effects of cigarette smoking are well established, this programme of work was designed with the novel aim of demonstrating whether smoking influences the development of physical fitness; what biological processes might be responsible; and if this occurs, where in the time-course of training this influence may be manifest. A possible reason for an apparent lack of previous research to this end is the inherent probability that a population seeking positive health and fitness outcomes is unlikely to habitually smoke, and that habitual smokers are unlikely to partake in, and indeed continue smoking through, standardised progressive training. Military trainees provide a relatively unique opportunity to examine a population with a potentially high proportion of smokers, and a high adherence to both the continuance of smoking, and to prolonged exercise training. Further rationale for the research was based on the lack of extensive understanding of smoking behaviour and injury risk in the British army training population. These studies include the first description of smoking habits and comprehensive analysis of injury incidence in the largest training establishment in the British Army. To provide basis for further central discussion, the main findings of completed studies are summarised below.

Study 1 (*Chapter 4*)

Aim: To describe smoking prevalence at Infantry Training Centre, Catterick (ITC(C)) and to give a more comprehensive understanding of the smoking behaviour of the British Army trainee population.

Key Findings:

- Regular smokers (>1 cig/day) comprised 48% of trainees on entry to training.

- Regular smokers averaged (mean (\pm SD)) 11.5 (\pm 5.8) cigarettes per day for 6.0 (\pm 3.3) years.
- 85% of trainees remained the same smoking status, and the majority of those that changed were former smokers recommencing regular smoking.

Study 2 (*Chapter 5*)

Aim: To examine whether smokers exhibit increased resting markers of oxidative stress and systemic inflammation, and altered hormone concentrations compared with non-smokers at entry to training, and whether greater differences would result from greater cigarette consumption.

Key Findings:

- Resting serum concentration of marker of oxidative stress malondialdehyde (MDA) was significantly higher in habitual smokers and each subgroup categorised by cigarette consumption in comparison to non-smokers.
- No significant differences in resting serum concentrations of CRP, IL-6, ALT, testosterone, cortisol or IGF-1 existed between smoking groups and non-smokers.

Study 3 (*Chapter 6*)

Aim: To examine whether training elicits different changes in resting markers of oxidative stress, systemic inflammation, hormone concentrations, physical performance variables and lower leg muscle characteristics in smokers compared to non-smokers during 10 weeks of military training.

Key Findings:

- Military training elicited significant improvement in the majority of performance measures, irrespective of group.
- Performance improvement was not significantly impaired in smokers in any performance measure when compared to non-smokers, and muscular adaptation did not differ between groups. Though not significant, a trend existed for greater improvement in run performance in non-smokers ($p=0.067$).

- Resting serum concentrations of MDA and CRP were significantly higher in habitual smokers than non-smokers, irrespective of training time. MDA, irrespective of group, significantly reduced during training. CRP was similar in smokers and non-smokers at baseline and increased to a greater extent in smokers during training.
- No differences between smokers and non-smokers existed in resting IL-6, ALT, testosterone, cortisol or IGF-1 during training, and no evidence of a dose-response existed on any biochemical marker.

Study 4 (*Chapter 7*)

Aim: To explore whether habitual smoking impairs improvement in performance of military physical fitness tests (PFT) during 26 weeks of initial training in a large sample of trainees.

Key Findings:

- No interaction effects occurred, demonstrating that improvement in performance did not differ between smokers and non-smokers in press up, sit up or run performance.
- In all performance measures, non-smokers performed significantly better irrespective of time.

Study 5 (*Chapter 8*)

Aim: To examine whether the responses of inflammatory markers and hormones to bouts of military exercise and to two days of simulated operational stress would differ between smokers and non-smokers.

Key Findings:

- No differences between smokers and non-smokers existed in responses to acute exercise or to two consecutive days of training in concentration of CRP, IL-6, testosterone, cortisol or IGF-1.
- The “10-miler” event elicited a greater IL-6 response than the “log race” event, likely due to greater duration.
- CRP was significantly increased at rest the day following the “10-miler”.

Study 6 (*Chapter 9*)

Aim: The aim of this study was to examine overall injury incidence and prevalence of training-related injuries specifically to the lower-limb and lumbar spine in the British infantry training population at ITC(C), and to investigate whether habitual smokers are at greater risk of training-related injury than non-smokers.

Key Findings:

- 58% of trainees sustained at least one training-related injury to the lower-limb and lumbar spine.
- Risk of training-related injuries and time-loss training injuries was significantly greater in smokers than non-smokers, and specifically in injuries attributed to overuse.
- Duty days lost to time-loss injury per 1000 trainee days was significantly higher in smokers than non-smokers.
- The most common injuries were non-specific soft tissue injury to the knee, ankle and foot.

This programme of work improved on previous research, first, by the measures employed to determine smoking behaviour using more precise definitions of smoking groups, and more accurately attaining data on smoking history and not solely current status. The administered questionnaire investigated years smoked, and distinguished between former and non-smokers, and occasional and regular smokers. These methods and those to ensure anonymity lend confidence to the finding that smoking prevalence (48%) in infantry training was higher than previously observed in other British training populations and in the British general public. By establishing prevalence and average tobacco exposure in a large cohort, Study 1 demonstrated that if the mechanistic rationale was accepted and adverse influences of smoking did exist in this training population that they would affect a substantial proportion of trainees. The findings of Study 1 give a value for average tobacco exposure (cigarette consumption rate and years smoked) in this population, which by means of comparison show that the smoking habits of each study sample are representative of the overall training population.

Perhaps the most unexpected finding from this series of studies was that smokers did not exhibit poorer development in physical performance than non-smokers, either in a battery of performance measures in 46 trainees over 10 weeks, or in physical fitness test performance in a large cohort over 26 weeks. The larger sample size, and therefore greater statistical power in Study 4, as well as control for physical activity indicates that the trend for greater improvement in run time ($p=0.067$) observed in non-smokers over 14 weeks (Study 3) did not occur across the full course duration. Previously, the comparison between the magnitude of performance improvement between smokers and non-smokers appears to have been examined in only one study (Hoad & Clay 1992), demonstrating significantly greater improvement in non-smokers. The study by Hoad and Clay (1992) lasted 26 weeks, and measured a variety of body weight strength exercises completed to failure and a 2.4 km run in British Army officer cadets. The tests employed are designed to test aerobic and muscular endurance, and included similar exercises to those in the PFT in the current studies. Given the course duration and similar fitness tests used, it is surprising that Study 4 did not present similar findings to those of Hoad and Clay (1992).

Taken together, the findings that smokers performed worse in PFT criteria in Study 4; the run time trend in Study 3; and the work of Hoad and Clay, and others (Ward et al. 2003; Al-Obaidi et al. 2004; Haddock et al. 2007) suggest that poorer performance in smokers may occur in endurance and muscular endurance tasks. This provides some support that smoking affects the performance of exercises that involve a high number of repeated contractions, possibly highlighting a mechanism involved in the modulation of muscular fatigue. This agrees with two studies that demonstrated that where maximum force of muscle contraction did not differ between smokers and non-smokers, fatigue resistance was reduced in smoking individuals (Morse et al. 2007; Wüst et al. 2008). The precise explanation for how smoking could limit resistance to fatigue and performance in endurance exercise could involve a vast array of mechanisms, several of which may not be discernible from the findings of this work. However, the accumulation of reactive oxygen species (ROS) have been implicated in the development of muscular fatigue (Moopanar & Allen 2005; Reid & Moylan 2011), and reduced lactate threshold (Aguiló et al. 2007). This is particularly noteworthy given that in the current

programme of work oxidative stress was elevated in smokers at rest (Studies 2 and 3). Additionally, Bassett & Howley (2000) described the provision of oxygen and oxygen carrying capacity of the blood as limiting factors in aerobic capacity. The chronic inhalation of carbon monoxide may reduce oxygen carrying capacity (Silverstein 1992), potentially explaining significantly larger but less abundant red blood cells observed in smokers in Study 2. Though not measured in this programme of work, smoking is also associated with decreased secretion of parathyroid hormone (PTH), which would reduce peripheral vasodilation (Iseki 1990; Mandsager, Brewer & Myatt 1994), potentially increasing blood pressure and limiting blood transport to muscles. Although the underlying mechanisms remain unclear, the current work gives evidence that a regular smoker may exhibit poorer muscular fatigue resistance in physical tasks similar to those that typify military fitness testing or military occupational tasks.

Where the findings of the programme of work and the mechanistic rationale do not coalesce, is there being no impairment in adaptation in smokers despite evidence of elevated resting oxidative stress and, during training, greater inflammation than non-smokers. The rationale for examining markers of oxidative stress, inflammation and hormones at rest during training alongside adaptation was that smokers have previously exhibited distinct levels of these markers from non-smokers, and that the systemic concentration of these markers could mediate aspects of adaptation to training. It was postulated that these chronic differences from non-smokers would disrupt the processes of adaptation within the muscle cell, impairing adaptation in smokers when compared to non-smokers. Study 2 sought to indicate the chronic physiological status of smokers at entry to training and demonstrated, via MDA concentrations, significantly greater oxidative stress in smokers. It was surprising that this was not accompanied by significantly elevated inflammation, given the established links between these processes (Helmersson et al. 2005; Peake, Suzuki & Coombes 2007). However, significantly higher CRP in smokers was subsequently observed in Study 3. Similar to Study 2, baseline levels of CRP were alike among smokers and non-smokers, and therefore the effect of smoking status appears to be a function of an exacerbated rise in inflammation in smokers when combined with training.

It would be reasonable that heightened inflammatory response to training in smokers may be caused by the presence of chronically elevated oxidative stress. Elevated CRP at weeks 5 and 10 of training was likely to be a result of recent physical training. In Study 5, the “10-miler” elicited such a response on resting CRP the day following exercise. Bloomer et al. (2007) showed a significantly greater oxidative response to exercise stimulus in smokers than non-smokers in young active men. Possessing elevated resting oxidative stress could be the precursor for an exacerbated oxidative response to a stimulus, and could have evoked the same in inflammation in smoking individuals in Study 3. This is supported by dietary intervention of antioxidants being shown to attenuate cytokine response to exercise (Fischer et al. 2004). In Study 5, as neither greater inflammation nor acute inflammatory responses in smokers were observed in either exercise bout, it may be that oxidative stress was not elevated in smokers in this sample, in contrast to those in Study 3. Trainees involved in Study 5 were at the pinnacle of parachute regiment training, where higher fitness standards are enforced, and were likely to be substantially fitter than trainees at week 10 in Study 3. In Study 3, MDA concentrations showed a significant decline from weeks 1-10, suggesting a reduction in oxidative stress may be an adaptation to prolonged training. This is supported by research showing redox sensitive cells become more proficient at modulating oxidative stress from repeated exercise (McArdle et al. 2001; McArdle, Spiers, et al. 2004; Ji 2007). Similarly, in trained individuals when compared to untrained, the presence of antioxidant protein content and cytoprotective heat shock proteins were significantly higher at rest and following exercise, suggesting more effective protective mechanisms via mediation of redox balance (Morton et al. 2008). As such, similar to the anti-inflammatory effect of long term exercise, the longer duration of training and greater physical fitness of trainees in Study 5 may have reduced resting oxidative stress resulting in similar levels between groups. The implications here are that in a young otherwise healthy population of smokers, achieving a high level of physical fitness may counteract elevated inflammation and oxidative stress, and subsequently attenuate the potentially greater inflammatory response to training stimuli.

The mechanistic rationale behind adaptation in the current studies relies on the link between circulatory markers and the actions within local tissue. Since the

initiation of this project the scientific domain has experienced growing debate surrounding the extent to which circulatory markers correlate with the environment within local tissue. The evidence that links inflammation and oxidative stress in the circulatory system centres around the association between elevated inflammation and oxidative stress in disease states (Federico et al. 2007; Peake et al. 2007; Lozovoy et al. 2011). Locally, the two are linked by the cellular interplay of redox balance with cytokine mediation via transcription factors (Kosmidou et al. 2002; Aoi et al. 2004; Close et al. 2005). The persistent elevation of both inflammation and oxidative stress within muscle has implications on blunting the adaptive signalling and promotion of protein synthesis and hypertrophy (Goodman 1994; Close et al. 2005; Haddad et al. 2005; Yamada, Tomiyama, et al. 2006). Yet muscle cross sectional area or total area in Study 3, which would be indicative of net myofibril hypertrophy, did not differ between smokers and non-smokers despite the potential effects of elevated systemic oxidative stress and inflammation.

It is well-established that oxidative stress is elevated within lung tissue and bronchoalveolar lavage from the acute act of smoking (McCrea et al. 1994; Faux et al. 2009; Barreiro et al. 2010), but it is not clear whether the increase in oxidative stress in the circulation of smokers is as a direct result of this (ie- the efflux of lipid peroxidation into the circulation from the lung tissue), or an increase in systemic oxidative stress as a result of the circulatory rise in inflammatory markers signalled by lung tissue, or a combination of both. Similarly, it is not certain whether a chronically elevated state of circulatory oxidative stress or inflammation will elicit a paralleled environment within the muscle cellular environment. This uncertainty may explain how the observation of significantly elevated circulatory oxidative stress and inflammation in smokers during training was not accompanied by any impaired muscular adaptation in smokers.

A similar debate exists in the relevance of circulatory versus local measurement of IGF-1 and testosterone, alongside whether there is any significance in shifts in endogenous hormones after maturation. With regard to testosterone, some uncertainty may be caused by assay variability and the definitions of when testosterone is biologically active. Examination of the methods for measuring testosterone has elucidated a number of differences in assays and blood sample timing that are pivotal for correctly determining testosterone concentrations (Gray

et al. 1991). The majority of circulatory testosterone (65-80%) is bound to sex hormone binding globulin (SHBG), and is therefore biologically inactive. Theoretically, the proportion of bioavailable testosterone is reduced by an increased circulating concentration of SHBG. However, some assays cause the unbinding of testosterone from SHBG and others from albumin, where some use calculations to estimate the bioavailable fraction. As such, despite apparent increases in SHBG, some studies have observed no significant differences in bioavailable testosterone (Field et al. 1994; English et al. 2001). Interestingly, although only measured in a handful of studies, SHBG has been shown to be elevated in smokers (Field et al. 1994; English et al. 2001; Svartberg et al. 2003). It is therefore entirely possible that smoking influences bioavailability of testosterone instead of the total concentration as measured in the current programme of work.

It is increasingly considered that the local production and action of IGF-1 within muscle has far greater implications for maintenance of muscle mass than hepatic production (Stewart & Pell 2010). While there is increasing concern that circulatory concentration of both IGF-1 (Criswell et al. 1998; Friedlander et al. 2001) and testosterone (Wilkinson et al. 2006; West et al. 2009) do not correlate with what occurs on a cellular level. The current scheme of work appears to support the growing consensus of work that suggests circulatory markers may not ultimately be concurrent with the effects observed in the presence of those same markers in cell lines. Given the debate, it may be that attempting to assess cellular effects of elevated IGF-1 is less relevant, certainly to the current work, than endogenous IGF-1 acting as an indicator of physiological or metabolic strain (Nindl, Alemany, et al. 2007; Nindl et al. 2011). In Study 3, a significant reduction in IGF-1 was observed following days with the largest training volume, while the implementation of arduous military exercise in Study 5 elicited a decline that approached significance ($p=0.055$). Although the decline in IGF-1 in neither of these studies was more pronounced in smokers, the wider implication for all trainees is that repeated days of training of this nature in quick succession without sufficient recovery is likely to elicit a severe state of metabolic stress.

Study 6 demonstrated a significantly greater incidence, and therefore risk, of training injury in smokers as well as those resulting in training time-loss, and specifically those attributed to overuse. Studies 2 to 5 demonstrated that greater

oxidative stress and inflammation in habitual smokers did not influence adaptation in muscle or changes in performance with training. However, these could, via a number of interlinked mechanisms, weaken the musculoskeletal system, increasing susceptibility to injury. Circulatory markers of oxidative stress have been shown to be negatively associated with bone mass (Basu et al. 2001) and ROS stimulate osteoclastic bone resorption (Garrett et al. 1990). The interactions of PTH and inflammatory markers, both affected by smoking, have been linked to the mediation of bone turnover (Lips 2001; Ragab et al. 2002; Swarthout et al. 2002). Additionally, chronic nicotine exposure has been shown to increase insulin resistance and impair muscle glycogen repletion (Price, Krishnan-Sarin & Rothman 2003). The plethora of possible biochemical effects of cigarette smoking are complex and it can be asserted that many other pathways for the long term effect of smoking on injury susceptibility exist in addition to those proposed in the current work. The specific observation of higher overuse injury and time-loss injury occurrence in smokers would suggest a long-term impairment of recovery of soft tissue and/or bone in comparison to non-smokers. This is supported by lower bone mass (Joseph, Kenny, et al. 2005; Wong et al. 2007) and collagen production (Jorgensen et al. 1998) in smokers, as well as increased stress fracture risk (Lappe et al. 2001). Though speculative, in relation to soft-tissue, it is possible that with less effective resorption-formation coupling of bone an excess of physical training could substantially increase physical strain on joints and supporting musculature, potentially increasing risk of muscular or connective tissue injury.

Greater resting inflammation in smokers, as evidenced from data in Study 3, could also have implications on recovery. This is particularly relevant given that a significantly higher number of duty days were lost per 1000 trainee days from time-loss injuries in smokers than non-smoking trainees. Elevated inflammation and oxidative stress observed in smokers may prolong injury recovery time or enhance severity of a present injury. Both chronically elevated inflammation (Barbe & Barr 2006) and the presence of MDA (Freeland et al. 2002) have been implicated in increased severity of injuries. It seems that the balance of neutrophils and macrophages, and indeed the subsequent modulation of oxidising agents, is integral to the process of tissue repair (Toumi & Best 2003), and as proposed with muscular adaptation, may be disrupted by systemic inflammation and redox balance. An

aspect of the research area also emerged in support of the premise that increased injury risk in smoking is likely to be multivariate, and not solely as an indirect result of the act of smoking. The observation of higher risk in light smokers than heavy smokers in some injury types suggests an absence of a dose-response, and that smoking itself is not the inciting factor in the mechanisms for higher risk. This highlights that a smoking population has a number of inherent factors whose interplay warrant examination. For instance, it is possible that poorer lifestyle choices such as lower dietary intake of nutritious foods, lower physical activity and participation in sport, and greater risk taking in smokers could affect both injury and recovery, such as via reduced adherence to rehabilitation or correct nutritional intake.

Discussion of methodological approach

The scheme of work completed for this thesis investigated several novel aspects of the influence of habitual smoking on young, physically active humans. Studies were designed with an aim to observe an accurate representation of the population in its real-world setting. Therefore, it is acknowledged that this thesis presents a series of explorative field-based studies, where the controlled experimental rigour of laboratory controlled trials, could not always be implemented.

Studies were designed such that they did not conflict with normal daily training and habitual routines of participants. It can therefore be assured that trainees examined in this research followed the same training regime as all British infantry trainees and, as such, present as an ecologically valid representation of the training population. It could also be suggested that the training environment itself acted, in part, similar to laboratory-based experimental control. Typically, dietary intake, waking hours and physical training will have been similar between trainees during each specific training week, and trainees would be confined to the training base for the majority of time. In a free-living study there would be greater concern that these factors may have been confounders to primary outcomes measures, such

as performance in physical tasks and potentially the levels of resting inflammation, oxidative stress and hormones. As such, it should be acknowledged that the use of a regimented training environment establishes substantially more control than some other free-living field-based research. It is clear, though, that extreme alterations in training environment or lifestyle would limit this control. As observed in military training programmes previously, severely restricted hours of wakefulness combined with consecutive days of intense physical work could produce responses in inflammation and hormones with high inter-individual variability (Booth et al. 2006; Alemany et al. 2008). Similarly, illness, timing of blood sample or hydration status could interfere with interpretation of biochemical marker analysis. Systemic concentrations in response to exercise can be influenced by infection or changes in plasma volume. Several of these factors could be improved upon in future work, but were not feasible currently without further disruption of the lifestyle of military trainees.

In the context of cigarette smoking, previous studies have reported several limitations to self-report smoking behaviour. In this programme of work, every effort was made to reduce these limitations, including security and anonymity measures to limit social bias, and tailoring questions to more accurately distinguish between smoking behaviours than solely smoking or never-smoking. One concern was that smoking status may drastically fluctuate during training, whereby self-report responses could be confounded by memory. However, where possible the follow-up questionnaire was used to ascertain the occurrence of changes in smoking status, and conflicting respondents removed. Equally, without social bias, there is little reason for a smoker to respond as non-smokers or vice versa. It is also noted that this research took place following the national smoking ban, meaning no smoking in working environments and included all military buildings on camp. As smoking trainees would be required to smoke in well-ventilated outdoor shelters, it is likely that environmental tobacco smoke or passive smoking was minimal.

In addition, it was a major concern that military drop-out from the population being examined was unavoidable. With regard to adaptation across training weeks, this indirectly reduced sample size and introduced potential bias towards those who may have responded more positively to military training, or not become injured. To counteract this, in Study 4, a modelling process was

implemented to use data from all participants at all time points, and did not markedly alter findings. Unfortunately, this process of analysis was not possible for studies 2 and 3 which used the same pool of trainees, with 110 (45 non-smokers, 65 smokers) at entry and 65 (24 non-smokers and 41 smokers) by week 10. Of those 41 smokers, only 24 completed the majority of physical performance tests, showing 17 smokers who may have altered findings if they had agreed to complete the final battery of tests. Despite these instances, the aim of the research, and potential impact, was to present the responses and outcomes of individuals in real-world training without greatly disrupting the lifestyle of trainees, and present the findings assuming those individuals are a representative sample of the population under examination. As with many field based and epidemiological studies, the aim is to achieve a large enough sample size to counteract the possible variation incited by lower experimental control. It is believed that not only did the programme of work achieve greater control than similar free-living examples, but that the current studies provided sufficient power to present externally valid findings for this population.

Future directions

Arguably, as military trainees are one of few populations that have a high proportion of individuals who habitually smoke while attempting to develop physical fitness, and that differences in training adaptation were not observed here, it may be there is no necessity for further work to show whether smoking elicits this effect. With this in mind, the implications for the military would be that being a smoker even in a young active population may increase risk of injury, elongate recovery from those injuries, and physical performance itself may be affected. It is also clear that oxidative stress and inflammation are aspects of the influence of smoking on the examined physiological status of smokers, highlighting a potentially adverse impact on health further into service.

It would be challenging to quantify the cumulative adverse effects of the alteration in selected biochemical markers observed in the current work, but given that both oxidative stress and inflammation are implicated in poorer health

outcomes, it may be that future work should try to determine whether these effects are reversible at different stages of life. The potential adverse effect of smoking and these baseline differences from non-smokers on health once trainees become servicemen could be a concern for military organisations in terms of economic cost and occupational effectiveness. However, the current programme of work does give some indication for there being a beneficial effect of long-term exercise training on the reduction of oxidative stress and inflammation without smoking cessation, certainly in a younger adult population. Longitudinally, the comparison of the health profiles of physically active smokers versus sedentary smokers could indicate how potent a protective measure exercise can be against some adverse effects of smoking. This would be with an aim to assess at what stages of life physical activity can be sufficient to counteract smoking-induced alterations in physiological potentially more beneficial to overall health.

On a more mechanistic level, any underlying mechanisms for an association between smoking and physical fitness, and on injury susceptibility, are still unknown. As discussed, the plethora of potential pathways for increased injury risk in smokers could highlight the need for the examination of many markers, possibly integrating biochemical markers of bone metabolism and soft tissue repair during the recovery phase of injury in smokers and non-smokers. In the context of fatigue, the interaction between basal levels of oxidative stress and inflammation and their responses to exercise with relation to rest are not understood. This may contain implications for both the short-term effects of exercise on habitual smokers and modulation of contractile fatigue. In a laboratory setting, specifically with controlled pre-trial diet, hydration and physical activity- and fitness-matched participants, a comparison of the oxidative and inflammatory responses to exercise, taking into account resting levels of these markers in a group of smokers and non-smokers would add to the work of Bloomer et al. (2007). This may provide insight into whether an elevated resting level of oxidative stress or inflammation as observed in chronic disease states might mediate an exacerbated response to exercise. As the area is still relatively unclear, a test for muscular fatigue resistance could be employed pre- and post- an exercise training intervention as this may be where smoking-induced oxidative stress is most influential. On a grander scale, these studies may begin to explain whether the chronic effects of smoking could

alter physiological responses to physical activity. If research managed to outline underlying mechanisms it may be that steps could be taken to protect from this, such as the involvement of oxidative stress being tempered by dietary antioxidant supplementation.

Of course, there is also a speculative discussion around the potential harm of smoking following training. If, as proposed by the current research, there is a relatively protective effect of exercise training on some adverse effects of smoking there is the added possibility that physical activity, and subsequently physical fitness, may reduce once trainees become serviceman. This is combined with the additional smoking exposure that may result from war deployment. If inflammatory markers and oxidative stress are elevated at rest from smoking and continue to increase with age, it may be that over a serviceman's career the health risks could be, ultimately, more serious in smokers than non-smokers. It would be of interest, then, to longitudinally study the inflammatory profile of servicemen who smoke and possibly eventual health outcomes.

From the data provided on increase injury risk, attenuated recovery and poorer overall fitness, it would appear to be prudent for the British military to maintain efforts in anti-smoking policy. From the current research, the health costs associated with greater injury prevalence, longer rehabilitation and more common occurrence of illness are not quantifiable, but may be a concern to military organisations. As the data suggests that being a smoker brings with it a combination of potentially harmful lifestyle choices, programmes encouraging smoking cessation and adherence to a healthy lifestyle may go some way to ensuring better training outcomes for personnel and health outcomes from service.

Conclusion

This series of studies demonstrated that any adverse effects of smoking could potentially influence a large proportion of military trainees. Habitual smokers exhibited elevated resting oxidative stress compared with non-smokers, and more pronounced inflammatory changes as a result of training, which may have possible implications on future health outcomes and recovery from injury. Habitual smoking, and differences in inflammation and oxidative stress as a result of smoking, did not appear to effect training adaptation. However, habitual smokers are likely to perform worse in physical tasks which require aerobic and muscular endurance, potentially mediated by reduced resistance to muscular fatigue. There was evidence that in a young, otherwise healthy smoking population, partaking in the full duration (26 weeks) of initial military training may counteract the adverse effects of smoking on elevated inflammation and oxidative stress. Additionally, although the mechanisms are unknown, habitual smokers are at a greater risk of training-related injury and injuries attributed to overuse, indicating a potentially attenuated recovery response to exercise training. Ultimately, despite the likelihood that injury risk in smokers is a function of many interlinking factors inherent with a smoking population and may not be solely caused by smoking *per se*, this work demonstrates a habitual smoker may exhibit poorer performance in military training and eventual training outcome.

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Appendices

Appendix A – Consent form used to accompany each study participant information sheet

CONSENT FORM



Project title

Risk factors for injury during British Army Infantry recruit training.

Ministry of Defence Research Ethics Committee Reference:

0805/160

Please read the following carefully and circle either ‘yes’ or ‘no’:

- The nature, aims and risks of the research have been explained to me. Yes / No
- I have read and understood the Participant Information Sheet. Yes / No
- I understand what is expected of me. Yes / No
- I understand that I can withdraw from the study at any time without giving a reason. Yes / No
- I understand that if I do withdraw it will not affect my Army career in any way. Yes / No
- I understand that participating in this study is not part of my Army training course or assessment. Yes / No
- I consent to the processing of my personal information for the purposes of this research study. Yes / No
- I understand that my information will be treated as strictly confidential in accordance with the Data Protection Act 1998. Yes / No
- I understand that Army staff will not have access to my results. Yes / No
- I am aware that my consent is specific to this study and this study only. Yes / No
- I agree to volunteer as a subject for the study described in the information sheet and I give full consent to my participation in this study. Yes / No

Participant’s Statement:

I (insert name)agree that the research project named above has been explained to me to my satisfaction and I agree to take part in the study. I have read both the notes written above and the Participant Information Sheet about the project and understand what the research study involves.

Signed **Date**

Witness Name Signature

FOR PROJECT STAFF USE ONLY

Investigator's Statement:

I (insert name) confirm that I have carefully communicated the nature, demands and any foreseeable risks (where applicable) of the proposed research to the participant.

Signed Date

Name and contact details of Independent Medical Officer (if appropriate):

Dr Mark Langham, Senior Medical Officer, Vimy Barracks, Infantry Training Centre, Catterick Garrison, Catterick. Phone: 01748 872653

Name and contact details of Principal Investigator:

Dr Keith Stokes
Lecturer in Human and Exercise Physiology
Sport and Exercise Science
School for Health
University of Bath

Appendix B – Military Pre-training Questionnaire



Lifestyle Questionnaire

Please be as **HONEST** and **ACCURATE** as possible.

Army staff **WILL NOT** have access to your responses.

Your participation in this study will not affect your career in any way and **IS NOT PART** of your course or assessment.

STRICTLY CONFIDENTIAL

How to fill in this questionnaire:

- A Most of the questions on the following pages can be answered by simply ticking **one** box below or alongside the answer that applies to you.

Example:

tick **ONE** box

Yes

No

Have you ever broken a bone?

☒
☐

- B Sometimes you are asked to write in a number or the answer in your own words. Please enter numbers as figures rather than words.

Example:

Write in how old you were

14

years old

- C **ON MOST PAGES YOU SHOULD ANSWER ALL QUESTIONS** but sometimes you will find the box you have ticked has an arrow next to it which tells you to go to another question.

By following the instructions carefully you will miss out questions which do not apply to you.

Example:

Yes

☐

No

☒

GO TO Q5-4

If you would rather not answer a question please clearly state 'NO COMMENT'

Please complete this section

☐

Guards

☐

Para

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Line

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Infantry Course:

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Service Number

FOLD

Complete section, fold as indicated, remove protective strip and stick down

SECTION 1: PHYSICAL ACTIVITY & EXERCISE	
Q1-1	<p>Before joining the Army, how active do you think you were compared with other men the same age as you?</p> <p style="text-align: right;">tick ONE box</p> <p style="text-align: right;"> <i>much less active</i> <input type="checkbox"/> <i>slightly less active</i> <input type="checkbox"/> <i>about the same</i> <input type="checkbox"/> <i>slightly more active</i> <input type="checkbox"/> <i>much more active</i> <input type="checkbox"/> </p>
Q1-2	<p>In a typical week before joining the Army, HOW MANY TIMES did you do the following kinds of exercise FOR MORE THAN 15 MINUTES during your free time?</p> <p>PLEASE WRITE A NUMBER IN EACH BOX</p> <p style="text-align: right;">write in number of times a week</p> <p>a) HARD, TIRING EXERCISE (heart beats rapidly and get out of breath quickly) (i.e. running, jogging, hockey, football, rugby, basketball, hard swimming, long distance cycling, hard weights session, karate)</p> <p style="text-align: right;"><input type="text"/> (e.g. 5)</p> <p>b) MODERATE EXERCISE (fairly tiring but not exhausting) (i.e. fast walking, tennis, easy cycling, badminton, easy swimming, volleyball, rock climbing, hill walking, easy weights session, gardening, cricket)</p> <p style="text-align: right;"><input type="text"/> (e.g. 0)</p> <p>c) MILD EXERCISE (small amount of effort) (i.e. bowling, yoga, golf, easy walking)</p> <p style="text-align: right;"><input type="text"/> (e.g. 7)</p>
Q1-3	<p>In a typical week before joining the Army, how often DURING YOUR SPARE TIME did you do any activity long enough to work up a sweat (heart beats quickly)?</p> <p>NEVER/RARELY <input type="checkbox"/> SOMETIMES <input type="checkbox"/> OFTEN <input type="checkbox"/></p> <p style="text-align: center;">(tick ONE box)</p>
Q1-4	<p>What type of exercise did you do the most before joining the army?</p> <p style="text-align: right;">tick ALL types of exercise you did</p> <p style="text-align: right;"> <i>I didn't exercise</i> <input type="checkbox"/> <i>running</i> <input type="checkbox"/> <i>team sport: football, hockey, rugby etc</i> <input type="checkbox"/> <i>swimming</i> <input type="checkbox"/> <i>cycling</i> <input type="checkbox"/> <i>weight training</i> <input type="checkbox"/> </p> <p>other (please give details) _____</p>

Q1-5	<p>In the 6 months before joining the Army how often did you complete a continuous run (steady pace)?</p> <p style="text-align: right;">tick ONE box</p> <p style="text-align: right;">never <input type="checkbox"/></p> <p style="text-align: right;">less than once a month <input type="checkbox"/></p> <p style="text-align: right;">1 - 3 times a month <input type="checkbox"/></p> <p style="text-align: right;">1 - 2 times a week <input type="checkbox"/></p> <p style="text-align: right;">2 - 4 times a week <input type="checkbox"/></p> <p style="text-align: right;">5 or more times a week <input type="checkbox"/></p>
Q1-6	<p>In the 6 months before joining the Army how much did you change the amount of exercise you did compared to the 6 months before that?</p> <p style="text-align: right;">tick ONE box</p> <p style="text-align: right;">increased a lot <input type="checkbox"/></p> <p style="text-align: right;">increased <input type="checkbox"/></p> <p style="text-align: right;">did not change <input type="checkbox"/></p> <p style="text-align: right;">decreased <input type="checkbox"/></p> <p style="text-align: right;">decreased a lot <input type="checkbox"/></p>
SECTION 2: INJURY HISTORY	
Q2-1	<p>Have you ever broken or fractured a bone(s)?</p> <p style="text-align: right;">tick ONE box</p> <p style="text-align: right;">yes <input type="checkbox"/> no <input type="checkbox"/></p> <p style="margin-top: 10px;">If yes, please give details _____</p> <p style="margin-top: 5px;">(which bone(s), left or right side, how old you were)</p>
Q2-2	<p>Have you ever had a lower leg stress fracture?</p> <p style="text-align: right;">tick ONE box</p> <p style="text-align: right;">yes <input type="checkbox"/> no <input type="checkbox"/> don't know <input type="checkbox"/></p> <p style="margin-top: 10px;">If yes, please give details _____</p> <p style="margin-top: 5px;">(left or right leg, how old you were)</p>

Q2-3	Have you ever suffered from shin pain?	tick ONE box	yes <input type="checkbox"/> no <input type="checkbox"/>		
	If yes, please give details _____ (left or right leg or both)				
Q2-4	In the past year have you suffered from any kind of injury that meant you couldn't take part in exercise or sport for longer than a week?	tick ONE box	yes <input type="checkbox"/> no <input type="checkbox"/>		
	If yes, please give details _____ (what kind of injury, how long you were out for)				
SECTION 3: DIET					
Q3-1 Please tick the box that best describes your eating habits BEFORE YOU JOINED THE ARMY:					
TOTAL	In an <u>AVERAGE WEEK</u>, how often did you:	Usually/ Often	Sometimes	Rarely/ Never	Does not apply to me
MEALS	1. Skip breakfast? 2. Eat <u>4 or more</u> meals from sit-down or take-out restaurants?	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	
GRAINS	3. Eat <u>more than 3 servings</u> of whole grain products a day? (Serving = 1 slice of 100% whole grain bread; 1 cereal bowl of whole grain cereal like Shredded Wheat, Weetabix, All-Bran, oatmeal, 3 to 4 whole grain crackers, ½ cereal bowl of brown rice or whole wheat pasta)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
FRUITS & VEGETABLES	4. Eat <u>more than 2-3 servings</u> of fruit a day? (Serving = ½ cereal bowl of small fruit (grapes, berries etc or 1 medium fruit or a glass of 100% fruit juice) 5. Eat <u>more than 3-4 servings</u> of vegetables/potatoes a day? (Serving = ½ cereal bowl of vegetables or potatoes, or 1 cereal bowl of leafy vegetables)	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	

TOTAL	In an <u>AVERAGE WEEK</u> , how often did you:	Usually/ Often	Sometimes	Rarely/ Never	Does not apply to me
DAIRY	<p>6. Eat or drink more than 2-3 servings of milk, yogurt, or cheese a day? Serving = ½ pint of milk or yogurt; matchbox size of cheese)</p> <p>7. Use semi-skimmed or whole milk instead of skimmed (no fat) milk?</p> <p>8. Use regular cheese like Brie, Cheddar, Red Leicester, Stilton instead of low fat or part skim cheeses as a snack, on sandwiches, pizza etc?</p>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<p>Do not use milk <input type="checkbox"/></p> <p>Do not eat cheese <input type="checkbox"/></p>
MEATS/CHICKEN/TURKEY	<p>9. Eat beef, pork, or dark meat chicken (leg and thigh)?</p> <p>10. Eat more than 170 grammes (see sizes below) of meat, chicken, turkey or fish per day? Note: 170 grammes of meat or chicken is the size of 2 decks of cards or the same as the following: 2 x regular hamburger 2 x chicken breast or leg (thigh and drumstick) 2 x pork chop</p> <p>11. Choose higher fat red meats like prime rib, T-bone steak, hamburger, ribs etc. instead of lean red meats?</p> <p>12. Eat the skin on chicken and turkey or the fat on meat?</p> <p>13. Use regular processed meats (like salami, corned beef, hotdogs, sausage, bacon) instead of low fat processed meats (like roast beef, turkey, lean ham, low-fat cold cuts/hotdogs)?</p>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<p>Do not eat meat, chicken, turkey or fish <input type="checkbox"/></p> <p>Do not eat meat <input type="checkbox"/></p> <p>Do not eat meat or poultry <input type="checkbox"/></p> <p>Do not eat processed meats <input type="checkbox"/></p>
FRIED FOODS	14. Eat fried foods such as fried chicken, fried fish, French fries or chips?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
SNACKS	15. Eat regular crisps, nachos, tortilla chips, crackers, popcorn, nuts instead of low-fat crisps or low-fat crackers?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Do not eat these snack foods <input type="checkbox"/>

TOTAL	In an <u>AVERAGE WEEK</u> , how often did you:	Usually/ Often	Sometimes	Rarely/ Never	Does not apply to me
FATS AND OILS	16. Use <u>regular salad dressing & mayonnaise</u> instead of low-fat or fat-free salad dressing and mayonnaise?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Do not use dressing/mayo <input type="checkbox"/>
	17. Add <u>butter, margarine or oil</u> to bread, potatoes, rice or vegetables at the table?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Do not cook <input type="checkbox"/>
	18. <u>Cook with oil, butter or margarine</u> instead of using non-stick sprays like Crisp 'n Dry or cooking without fat?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
SWEETS	19. Use <u>regular sweets</u> like cake, cookies, pastries, doughnuts, muffins, and chocolate instead of <u>low fat or fat-free</u> sweets?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Do not eat sweets <input type="checkbox"/> Do not eat frozen desserts <input type="checkbox"/>
	20. Eat <u>regular ice cream</u> instead of low-fat or fat-free ice cream, frozen yogurt etc?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	21. Eat sweets like cakes, cookies, pastries, donuts, muffins, chocolate and jelly sweets more than 2 times per day ?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
SOFT DRINKS	22. <u>Drink 500 ml or more of</u> non-diet fizzy drinks, such as Coke or Lemonade, or fruit drinks, such as Sunny Delight, a day? Note: 1 can of fizzy drink = 330 ml	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
SALT	23. Eat high salt <u>processed foods</u> like canned soup or pasta, frozen/package meals (TV/microwave dinners etc), chips?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
	24. <u>Add salt</u> to foods during cooking or at the table?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

Q3-2 Do you regularly take any supplements? tick **ONE** box

yes ☐ no ☐

(if YES, tick **ALL** that apply) ←

combined multivitamin and mineral supplement	<input type="checkbox"/>	please give details
individual vitamin supplement (e.g. vitamin C, D)	<input type="checkbox"/>	please give details
individual mineral supplement (e.g. iron, calcium)	<input type="checkbox"/>	please give details
fish oils (e.g. cod liver oil)	<input type="checkbox"/>	please give details
protein	<input type="checkbox"/>	
creatine	<input type="checkbox"/>	
other	<input type="checkbox"/>	please give details

SECTION 4: DRINKING							
Q4-1	How often did you have a drink containing alcohol in the past year? <div style="border: 1px solid black; padding: 5px; font-size: 0.8em;"> Consider a drink to be a ½ pint of cider, beer or lager, a shot of a spirit (e.g. vodka, gin, rum etc), a glass of wine, a bottle of alcopop. </div>	<i>never</i>	<i>monthly or less</i>	<i>2 - 4 times a month</i>	<i>2 - 3 times a week</i>	<i>4 or more times a week</i>	
		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
		(tick ONE box)					
Q4-2	How many drinks did you usually have on a typical day/night when you were drinking in the past year?	<i>0</i>	<i>1 or 2</i>	<i>3 or 4</i>	<i>5 or 6</i>	<i>7 or 9</i>	<i>10 or more</i>
		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
		(tick ONE box)					
Q4-3	How often do you have six or more drinks on one occasion?	<i>never</i>	<i>less than monthly</i>	<i>monthly</i>	<i>weekly</i>	<i>more than once a week</i>	
		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
		(tick ONE box)					
SECTION 5: SMOKING							
GENERAL							
Q5-1	Have you <u>EVER</u> smoked a cigarette (including roll-ups)?						
	yes <input type="checkbox"/>	→	GO TO Q5-2	no <input type="checkbox"/>	→	END OF QUESTIONNAIRE	
Q5-2	How old were you when you first tried smoking a cigarette (including roll-ups) even if it was only a puff or two?					<i>write in how old you were</i> <div style="border: 1px solid black; width: 60px; height: 20px; margin-top: 5px;"></div>	
Q5-3	Do you smoke cigarettes <u>AT ALL</u> now (even if only occasionally)?						
	yes <input type="checkbox"/>	→	GO TO Q5-4	no <input type="checkbox"/>	→	GO TO Q5-11	

Q5-4 How often do you smoke <u>AT THE MOMENT</u>?											
<i>regularly - at least one cigarette/roll-up a day</i>	<input type="checkbox"/> → GO TO Q5-5										
<i>occasionally - less than one cigarette/roll-up a day</i>	<input type="checkbox"/> → GO TO Q5-16										
CURRENT SMOKERS											
Q5-5 For how many years have you been smoking cigarettes (including roll-ups) on a <u>REGULAR BASIS</u>?	<i>write in how many years</i> <div style="border: 1px solid black; width: 100px; height: 20px; margin: 5px auto; text-align: center; font-size: 0.8em;">years</div>										
Q5-6 Please rate your addiction to cigarettes on a scale of 0-100: 0 = I am NOT addicted to cigarettes at all 100 = I am VERY addicted to cigarettes	<i>write in how addicted you are</i> <div style="border: 1px solid black; width: 100px; height: 20px; margin: 5px auto;"></div>										
Q5-7 How many cigarettes do you usually smoke a day?	<i>write in number smoked a day</i> <div style="border: 1px solid black; width: 100px; height: 20px; margin: 5px auto;"></div>										
Q5-8 How soon after waking up do you usually smoke your first cigarette or roll-up?	<table style="width: 100%; text-align: center; font-size: 0.8em;"> <tr> <td><i>within first 5 minutes</i></td> <td><i>5 - 15 min</i></td> <td><i>15 - 30 min</i></td> <td><i>30 min - 1 hour</i></td> <td><i>1 hour or more</i></td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> </table> <p style="text-align: center; font-size: 0.7em;">(tick ONE box)</p>	<i>within first 5 minutes</i>	<i>5 - 15 min</i>	<i>15 - 30 min</i>	<i>30 min - 1 hour</i>	<i>1 hour or more</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>within first 5 minutes</i>	<i>5 - 15 min</i>	<i>15 - 30 min</i>	<i>30 min - 1 hour</i>	<i>1 hour or more</i>							
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>							
Q5-9 For you, quitting smoking for good would be....	<p style="text-align: right; font-size: 0.8em;">tick ONE box</p> <table style="width: 100%;"> <tr><td style="text-align: right;"><i>very easy</i></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td style="text-align: right;"><i>fairly easy</i></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td style="text-align: right;"><i>fairly difficult</i></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td style="text-align: right;"><i>very difficult</i></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td style="text-align: right;"><i>impossible</i></td><td style="text-align: center;"><input type="checkbox"/></td></tr> </table>	<i>very easy</i>	<input type="checkbox"/>	<i>fairly easy</i>	<input type="checkbox"/>	<i>fairly difficult</i>	<input type="checkbox"/>	<i>very difficult</i>	<input type="checkbox"/>	<i>impossible</i>	<input type="checkbox"/>
<i>very easy</i>	<input type="checkbox"/>										
<i>fairly easy</i>	<input type="checkbox"/>										
<i>fairly difficult</i>	<input type="checkbox"/>										
<i>very difficult</i>	<input type="checkbox"/>										
<i>impossible</i>	<input type="checkbox"/>										
Q5-10 How much do you agree with this statement? <i>"After a few hours without smoking I have a strong urge to have a cigarette"</i>	<p style="text-align: right; font-size: 0.8em;">tick ONE box</p> <table style="width: 100%;"> <tr><td style="text-align: right;"><i>totally disagree</i></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td style="text-align: right;"><i>fairly disagree</i></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td style="text-align: right;"><i>don't agree or disagree</i></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td style="text-align: right;"><i>fairly agree</i></td><td style="text-align: center;"><input type="checkbox"/></td></tr> <tr><td style="text-align: right;"><i>fully agree</i></td><td style="text-align: center;"><input type="checkbox"/></td></tr> </table>	<i>totally disagree</i>	<input type="checkbox"/>	<i>fairly disagree</i>	<input type="checkbox"/>	<i>don't agree or disagree</i>	<input type="checkbox"/>	<i>fairly agree</i>	<input type="checkbox"/>	<i>fully agree</i>	<input type="checkbox"/>
<i>totally disagree</i>	<input type="checkbox"/>										
<i>fairly disagree</i>	<input type="checkbox"/>										
<i>don't agree or disagree</i>	<input type="checkbox"/>										
<i>fairly agree</i>	<input type="checkbox"/>										
<i>fully agree</i>	<input type="checkbox"/>										
<div style="border: 1px solid black; padding: 5px; display: inline-block;"> Current Smokers: End of Questions </div>											

THOSE WHO HAVE TRIED SMOKING/EX-SMOKERS/OCCASIONAL SMOKERS (current regular smokers leave the rest of this page blank and go to section 6)					
Q5-11 Did you use to smoke regularly (at least 1 cigarette/roll-up a day)?	tick ONE box yes <input type="checkbox"/> no <input type="checkbox"/> → GO TO Q5-16				
Q5-12 For how many years did you smoke cigarettes (including roll-ups) on a <u>REGULAR BASIS</u> ?	<i>write in how many years</i> <div style="border: 1px solid black; width: 100px; height: 20px; margin-top: 5px; text-align: center; font-size: 0.8em;">years</div>				
Q5-13 How many cigarettes (including roll-ups) did you use to smoke a day?	<i>write in number smoked a day</i> <div style="border: 1px solid black; width: 100px; height: 20px; margin-top: 5px;"></div>				
Q5-14 How old were you when you <u>STARTED</u> smoking cigarettes (including roll-ups) on a <u>REGULAR BASIS</u> ?	<i>write in how old you were</i> <div style="border: 1px solid black; width: 100px; height: 20px; margin-top: 5px;"></div>				
Q5-15 Roughly when did you <u>STOP</u> smoking cigarettes (including roll-ups) on a <u>REGULAR BASIS</u> ?	<i>less than 1 week ago</i> <input type="checkbox"/>	<i>1 week - 1 month ago</i> <input type="checkbox"/>	<i>1 - 6 months ago</i> <input type="checkbox"/>	<i>6 - 12 months ago</i> <input type="checkbox"/>	<i>more than 1 year ago</i> <input type="checkbox"/>
(tick ONE box)					
Q5-16 When was the last time you smoked a cigarette (including roll-ups)?	<i>less than 1 week ago</i> <input type="checkbox"/>	<i>1 week - 1 month ago</i> <input type="checkbox"/>	<i>1 - 6 months ago</i> <input type="checkbox"/>	<i>6 - 12 months ago</i> <input type="checkbox"/>	<i>more than 1 year ago</i> <input type="checkbox"/>
(tick ONE box)					
End of questionnaire. Many thanks for your time.					

Appendix C - Log Transforms and back-transforms for determination of 95% confidence intervals (CI) in risk, odds and rate ratio of injury performed in Study 6 (Chapter 9)

Log transform for standard error (SE) and 95% CI for risk and odds ratio.

Log-transformed SE was calculated as follows:

$$SE_{(\log RR)} = \sqrt{\left[\frac{1}{Injured_{EXP}} - \frac{1}{n_{EXP}} + \frac{1}{Injured_{CON}} - \frac{1}{n_{CON}} \right]}$$

Where log RR is the natural log of risk or odds ratio, $SE_{(\log RR)}$ is the standard error of log RR, n is the group sample size, *EXP* is the exposure group (Smokers) and *CON* is the control group (Non-smokers).

Confidence interval was back-transformed using the following formula:

$$CI_{RR} = \frac{RR}{e^{[1.96 \times SE_{\log RR}]}} \text{ and } RR \times e^{[1.96 \times SE_{\log RR}]}$$

Log transform for SE and 95% CI for rate ratio.

$$SE_{Log \text{ Rate ratio}} = \sqrt{\frac{1}{Injured_{EXP}} + \frac{1}{Injured_{CON}}}$$

Confidence interval was back-transformed using the following formula:

$$CI = \frac{Rate \text{ ratio}}{e^{[SE \text{ of } \log [rate \text{ ratio}]]}} \text{ and } Rate \text{ ratio} \times e^{[SE \text{ of } \log [Rate \text{ ratio}]]}$$

Appendix D – Full blood count data from Study 2 (Chapter 5) organised by smoking sub-group.

Variable	Normative Range	Smoking Status	
		NS (n=36)	S (n=57)
(HB) Haemoglobin (g/dl)	13-18	15.3 (±0.2)	<i>LS</i> 15.1 (±0.3)
			<i>MS</i> 15.3 (±0.2)
			<i>HS</i> 15.1 (±0.3)
WBC (10 ⁹ /l)	4-11.0	8.2 (±0.3)	<i>LS</i> 8.1 (±0.5)
			<i>MS</i> 8.4 (±0.3)
			<i>HS</i> 9.0 (±0.9)
(PLT) Platelets (10 ⁹ /l)	150-400	257 (±8)	<i>LS</i> 253 (±16)
			<i>MS</i> 273 (±10)
			<i>HS</i> 284 (±31)
RBC (10 ¹² /l)	4.5-6.5	5.2 (±0.1)	<i>LS</i> 5.0 (±0.1)
			<i>MS</i> 5.1 (±0.1)
			<i>HS</i> 5.0 (±0.1)
(HCT) haematocrit (%)	0.40-0.54	0.43 (±0.0)	<i>LS</i> 0.42 (±0.01)
			<i>MS</i> 0.43 (±0.00)
			<i>HS</i> 0.43(±0.01)
(MCV) Mean corpuscular volume (fl)	78-98	82.5 (±0.4)	<i>LS</i> 84.0 (±0.8)
			<i>MS</i> 84.7 (±0.7)*
			<i>HS</i> 84.9 (±1.1)
(MCH) Mean corpuscular haemoglobin (pg)	27.5-32.5	29.7(±0.2)	<i>LS</i> 30.4 (±0.2)
			<i>MS</i> 30.1 (±0.2)
			<i>HS</i> 30.2 (±0.5)
Neutrophils (10 ⁹ ·L ⁻¹)	2-7.5	4.1 (±0.3)	<i>LS</i> 4.3 (±0.4)
			<i>MS</i> 4.3 (±0.2)
			<i>HS</i> 4.8 (±0.7)
Lymphocytes (10 ⁹ ·L ⁻¹)	1.5-4.0	2.9 (±0.2)	<i>LS</i> 2.6 (±0.2)
			<i>MS</i> 2.9 (±0.1)
			<i>HS</i> 3.0 (±0.3)
Monocytes (10 ⁹ ·L ⁻¹)	0.2-0.8	0.65 (±0.03)	<i>LS</i> 0.66 (±0.05)
			<i>MS</i> 0.70 (±0.04)
			<i>HS</i> 0.73 (±0.07)
Eosinophils (10 ⁹ ·L ⁻¹)	0.0-0.4	0.30 (±0.05)	<i>LS</i> 0.25 (±0.33)
			<i>MS</i> 0.27 (±0.03)
			<i>HS</i> 0.21 (±0.04)
Basophils (%)	0.0-0.2	0.58 (±0.12)	<i>LS</i> 0.74 (±0.29)
			<i>MS</i> 0.46 (±0.12)
			<i>HS</i> 0.43 (±0.16)

Note: Values are Mean (Standard Error). Subgroups are light smokers (LS; 1-9 cigarettes per day; n=16), moderate smokers (MS; 10-19 cigarettes per day; n=29) and heavy smokers (HS; ≥20 cigarettes per day; n=12). * indicates MCV is significantly higher in MS than NS (p=0.04).